



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12N 15/12, C07K 13/00, C12N 15/62, A61K 37/02, C12P 21/08	A1	(11) International Publication Number: WO 95/00641 (43) International Publication Date: 5 January 1995 (05.01.95)
(21) International Application Number: PCT/US94/06931 (22) International Filing Date: 17 June 1994 (17.06.94) (30) Priority Data: 08/079,510 17 June 1993 (17.06.93) US (71) Applicant: XOMA CORPORATION [US/US]; 2910 Seventh Street, Berkeley, CA 94710 (US). (72) Inventors: GAZZANO-SANTORO, Helene; Apartment 302, 4600 Susan Drive, San Bruno, CA 94066 (US). THEOFAN, Georgia; 3014 Sonoma Street, Torrance, CA 90503 (US). TROWN, Patrick, W.; 4057 Sugar Maple Drive, Danville, CA 94506 (US). (74) Agent: SHARP, Jeffrey, S.; Marshall, O'Toole, Gerstein, Murray & Borun, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606-6402 (US).		(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: LIPOPOLYSACCHARIDE BINDING PROTEIN DERIVATIVES (57) Abstract <p>Disclosed are novel biologically active lipopolysaccharide binding protein (LBP) derivatives including LBP derivative hybrid proteins which are characterized by the ability to bind to and neutralize LPS and which lack the CD14-mediated immunostimulatory properties of holo-LBP.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

LIPOPOLYSACCHARIDE BINDING PROTEIN DERIVATIVES

This is a continuation-in-part of U.S. Patent Application
Serial No. 08/079,510 filed June 17, 1993.

5

BACKGROUND OF THE INVENTION

The present invention relates generally to proteins useful for the treatment of gram-negative bacterial infections and specifically to the neutralization of the effects of lipopolysaccharide (LPS) which is also known as endotoxin. LPS is a major component of the outer membrane of gram-negative bacteria and consists of serotype-specific O-side chain polysaccharides linked to a conserved region of core oligosaccharide and lipid A. LPS is an important mediator in the pathogenesis of septic shock and is one of the major causes of death in intensive-care units in the United States. It has been observed that exposure to LPS during sepsis stimulates an immune response in monocytes and macrophages that results in a toxic cascade resulting in the production of tumor necrosis factor (TNF) and other proinflammatory cytokines. Morrison and Ulevitch, *Am. J. Pathol.*, 93:527 (1978). Endothelial damage in sepsis probably results from persistent and repetitive inflammatory insults. Bone, *Annals Int. Med.* 115:457 (1991).

LPS-binding proteins have been identified in various mammalian tissues. Among the most extensively studied of the LPS-binding proteins is bactericidal/permeability-increasing protein (BPI), a basic protein found in the azurophilic granules of polymorphonuclear leukocytes. Human BPI protein has been isolated from polymorphonuclear neutrophils (PMNs) by acid extraction combined with either ion exchange

25

chromatography or *E. coli* affinity chromatography. Weiss et al., *J. Biol. Chem.*, 253:2664 (1978); Mannion et al., *J. Immunol.* 142:2807 (1989).

The holo-BPI protein isolated from human PMNs has potent bactericidal activity against a broad spectrum of gram-negative bacteria.

5 This antibacterial activity appears to be associated with the amino terminal region (i.e. amino acid residues 1-199) of the isolated human holo-BPI protein. In contrast, the C-terminal region (i.e. amino acid residues 200-456) of the isolated holo-BPI protein displays only slightly detectable antibacterial activity. Ooi et al., *J. Exp. Med.*, 174:649 (1991). Human DNA
10 encoding BPI has been cloned and the amino acid sequence of the encoded protein has been elucidated. Gray et al., *J. Biol. Chem.*, 264:9505 (1989). Amino-terminal fragments of BPI include a natural 25 Kd fragment and a recombinant 23 Kd, 199 amino acid residue amino-terminal fragment of the human BPI holoprotein referred to as rBPI₂₃. See, Gazzano-Santoro et al.,
15 *Infect. Immun.* 60:4754-4761 (1992). In that publication, an expression vector was used as a source of DNA encoding a recombinant expression product (rBPI₂₃) having the 31-residue signal sequence and the first 199 amino acids of the N-terminus of the mature human BPI, as set out in SEQ ID NOS: 11 and 12 taken from Gray et al., *supra*, except that valine at
20 position 151 is specified by GTG rather than GTC and residue 185 is glutamic acid (specified by GAG) rather than lysine (specified by AAG). Recombinant holoprotein referred to herein as rBPI has also been produced having the sequence set out in SEQ ID NOS: 11 and 12 taken from Gray et al., *supra*, with the exceptions noted for rBPI₂₃. See also, Elsbach et al.,
25 U.S. Patent No. 5,198,541 the disclosure of which is hereby incorporated by reference. In addition to its bactericidal effects, BPI has been shown to neutralize the toxic and cytokine-inducing effects of LPS to which it binds.

Lipopolysaccharide binding protein (LBP) is a 60 kD glycoprotein synthesized in the liver which shows significant structural homology with BPI. Schumann et al. disclose the amino acid sequences and encoding cDNA of both human and rabbit LBP. Like BPI, LBP has a binding site for lipid A and binds to the LPS from rough (R-) and smooth (S-) form bacteria. Unlike BPI, LBP does not possess significant bactericidal activity, and it enhances (rather than inhibits) LPS-induced TNF production. Schumann et al., *Science*, 249:1429 (1990). Thus, in contrast to BPI, LBP has been recognized as an immunostimulatory molecule. See, e.g., Seilhamer, PCT International Application WO 93/06228 which discloses a variant form of LBP which it terms LBP- β .

One of the normal host effector mechanisms for clearance of bacteria involves the binding to and subsequent phagocytosis by neutrophils and monocytes. As part of this process, bacteria are exposed to bactericidal and bacteriostatic factors, including oxygen radicals, lysosomal enzymes, lactoferrin and various cationic proteins. LBP opsonizes LPS-bearing particles and intact Gram-negative bacteria, mediating attachment of these LBP-coated particles to macrophages. Wright et al., *J. Exp. Med.* 170:1231 (1989). The attachment appears to be through the CD14 receptor of monocytes which binds complexes of LPS and LBP. Wright et al., *Science* 249:1431 (1990). Anti-CD14 mAbs have been shown to block the synthesis of TNF by whole blood incubated with LPS. Wright et al. *Science* 249:1431 (1990). Interaction of CD14, which is present on the surface of polymorphonuclear leukocytes as well as monocytes, with LPS in the presence of LBP has been shown to increase the adhesive activity of neutrophils. Wright et al., *J. Exp. Med.* 173:1281 (1991), Worthen et al., *J. Clin. Invest.* 90:2526 (1992). Thus, while BPI has been shown to be

cytotoxic to bacteria and to inhibit proinflammatory cytokine production stimulated by bacteria, LBP promotes bacterial binding to and activation of monocytes through a CD14-dependent mechanism.

5 LPS, either directly or by inducing proinflammatory cytokines such as IL-1 and TNF, induces the expression of adhesion molecules including CD54 (intercellular adhesion molecule-1, ICAM-1) and E-selectin (endothelial-leukocyte adhesion molecule-1, ELAM-1) on endothelial cells, and thereby increases binding of leukocytes *in vitro*. Schleimer and Rutledge, *J. Immunol.* 136:649 (1986); Pohlman et al., *J.*
10 *Immunol.* 136:4548 (1986); Bevilacqua et al., *J. Clin. Invest.* 76:2003 (1985); Gamble et al., *Proc. Natl. Acad. Sci. USA.* 82:8667 (1985); Smith et al., *J. Clin. Invest.* 82:1746 (1988); and Bevilacqua et al., *Proc. Natl. Sci. USA* 84:9238 (1987). However, as CD14 has not been detected on the surface of endothelial cells (Beekhuizen et al., *J. Immunol.* 147:3761
15 (1990)) and no other receptor for LPS on endothelial cells has been identified, a different mechanism may exist whereby LPS can affect the endothelium.

Soluble CD14, found in serum (Bazil et al., *Eur. J. Immunol.* 16:1583 (1986)), has been hypothesized to be responsible for
20 transmitting the LPS signal to endothelial cells. Specifically, soluble CD14 has been shown to mediate a number of LPS-dependent effects on endothelial cells, including E-selectin and VCAM expression, IL-1, IL-6 and IL-8 secretion, and cell death. Frey et al., *J. Exp. Med.* 176:1665 (1992); Pugin et al., *Proc. Natl. Acad. Sci. USA.* 90:2744 (1993).

25 Recent studies have shown that soluble CD14 is involved in the LPS-mediated adhesion of neutrophils to endothelial cells. Anti-CD14 mAbs were able to completely inhibit the adhesion induced by LPS,

indicating that the contribution of other CD14-independent LPS receptors to these effects is minimal. The protein(s) on the endothelial cells that soluble CD14 might associate with to transduce the LPS signal remains to be identified. LBP has been shown to be involved in the signal transduction of LPS through soluble CD14; however, at high concentrations of LPS or soluble CD14, LBP does not further enhance the response of endothelial cells to LPS (Pugin et al., *Proc. Natl. Acad. Sci. USA.* 90:2744 (1993).

Larrick et al., *Biochem. and Biophysical Res. Commun.*, 179:170 (1991) relates to a cationic protein obtained from rabbit granulocytes which is identified as CAP18. CAP18 is identified as bearing no sequence homology with either BPI or LBP. In the course of their disclosure, Larrick et al. characterize other publications which discuss the structure of proteins including LBP and incorrectly attribute to the Wright et al., *supra* disclosure the speculation that "LBP is believed to be composed of two regions: an amino-terminal domain that binds to LPS and a carboxy-terminal domain that may (emphasis supplied) mediate binding of the LBP-LPS complex to the CD14 receptor on leukocytes."

Ulevitch, PCT International Application WO 91/01639 discloses methods and compositions for treatment of sepsis comprising administering anti-CD14 antibodies. The published application also describes "LBP peptide analogs" at page 17 which are stated to be polypeptides capable of competitively inhibiting the binding of LPS-LBP complexes to CD14 expressed on the surface of monocyte derived macrophages. The sequences of the three disclosed "LBP peptide analogs" show 90 to 100% homology with CD14 polypeptide sequences and no homology with LBP sequences.

Ulevitch et al., U.S. Patent No. 5,245,013 discloses a lipopolysaccharide binding protein which binds to Gram-negative bacterially secreted LPS and retards *in vitro* binding of LPS to high density lipoprotein.

5 Marra, PCT International Application WO 92/03535 discloses various chimeric BPI molecules including an rLBP/BPI chimeric molecule designated LBP25K/BPI30K [LBP(1-197)/BPI(200-456)] and comprising the first 197 amino acid residues of LBP and amino acid residues 200-456 of BPI wherein the coding sequence for the amino-
10 terminal 25 kD portion of LBP was linked to the coding sequence for the carboxy-terminal portion of the BPI protein by virtue of an engineered *Cla*I site within the coding sequence. The resulting molecule reacted positively in an ELISA assay utilizing anti-BPI protein antibodies and also reacted positively in an endotoxin binding assay. Rogy et al., *J. Clin. Immunol.*,
15 14: 120-133 (1994) describes experiments utilizing the LBP(1-197)/BPI(200-456) molecule wherein animals treated with the molecule in a primate bacteremia model demonstrated decreased LPS levels compared to controls, but still developed the sequelae of septic shock. For example, no significant reduction in endotoxin mediated cytokine synthesis was observed
20 in endotoxin-treated baboons to whom the compound was administered.

There exists a need in the art for LPS binding and neutralizing proteins which lack CD14-mediated immunostimulatory properties, including the ability to mediate LPS activity through the CD14 receptor.

SUMMARY OF THE INVENTION

The present invention provides novel biologically active polypeptide derivatives of Lipopolysaccharide Binding Protein (LBP), including LBP derivative hybrid proteins, which are characterized by the ability to bind to LPS and which lack CD14-mediated immunostimulatory properties, including the ability of LBP holoprotein to mediate LPS activity via the CD14 receptor. More particularly, LBP protein derivatives including LBP derivative hybrid proteins according to the invention lack those carboxy terminal-associated elements characteristic of the LBP holoprotein which enable LBP to bind to and interact with the CD14 receptor on monocytes and macrophages so as to provide an immunostimulatory signal to monocytes and macrophages.

Presently preferred LBP protein derivatives are characterized by a molecular weight less than or equal to about 25 kD. Particularly preferred LBP protein derivatives of the invention are LBP fragments comprising an amino-terminal region of LBP (e.g., amino acid residues 1-197). A molecule comprising the first 197 amino terminal residues of LBP and designated rLBP₂₅ exemplifies the derivatives of the invention. This particular derivative includes amino acid regions comprising LBP residues 17 through 45; 65 through 99 and 141 through 167 which correspond to respective LPS binding domains (e.g., residues 17 through 45, 65 through 99 and 142 through 169) of Bactericidal/Permeability-Increasing protein (BPI).

LBP derivative hybrid proteins of the invention comprise hybrids of LBP protein sequences with the amino acid sequences of other polypeptides and are also characterized by the ability to bind to LPS and the absence of CD14-mediated immunostimulatory properties. Such hybrid

proteins can comprise fusions of LBP amino-terminal fragments with polypeptide sequences of other proteins such as BPI, immunoglobulins and the like. Preferred LBP/BPI hybrids of the invention comprise at least a portion (i.e., at least five consecutive amino acids and preferably ten or more amino acids) of an LPS binding domain of BPI. One preferred LBP derivative hybrid protein of the LBP/BPI type comprises an amino-terminal LBP amino acid sequence selected from within the amino terminal half of LBP (e.g. within amino acid residues 1-197 of LBP) in which one or more portions of that sequence is replaced by the corresponding sequence of BPI selected from within the amino terminal half of BPI (e.g., within amino acid residues 1-199 of BPI). Another preferred LBP derivative hybrid protein comprises a fusion of amino terminal portions of LBP and heavy chain regions of IgG. Other LBP derivative hybrid proteins comprise LBP amino acid sequences into which all or portions of LPS binding domains of e.g., BPI or other LPS binding protein have been inserted or substituted for all or part of an LPS binding region of LBP. Preferred LBP derivative hybrid proteins include those in which all or portions of the previously-noted amino terminal LPS binding domains of BPI replace the corresponding region within LBP.

LBP protein derivatives and LBP derivative hybrid proteins of the invention are expected to display one or more advantageous properties in terms of pharmacokinetics, LPS binding, LPS neutralization and the like.

The present invention further provides novel pharmaceutical compositions comprising the LBP protein derivatives and LBP derivative hybrid proteins along with pharmaceutically acceptable diluents, adjuvants, and carriers and correspondingly addresses the use of LBP protein

derivatives and LBP derivative hybrid proteins in the manufacture of medicaments for treating gram negative bacterial infections and the sequelae thereof.

Polypeptides of the invention may be synthesized by
5 assembly of amino acids. In addition, the invention provides DNA sequences, plasmid vectors, and transformed cells for producing the LBP protein derivatives and LBP hybrid derivative proteins of the invention.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the
10 following detailed description of the invention which describes presently preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the DNA sequence and translated amino
15 acid sequence (1-197) of rLBP₂₅ [SEQ ID NOS:1 and 2];

Figure 2 depicts the DNA sequences and translated amino acid sequence (1-456) of rLBP [SEQ ID NOS:3 and 4];

Figure 3 depicts the construction of an rLBP₂₅ mammalian expression vector pING4505;

20 Figure 4 depicts construction of a vector pIC111 encoding an LBP (1-43)/BPI (44-199) hybrid protein;

Figure 5 depicts construction of mammalian expression vectors pING4525 and pING4526 for LBP/BPI hybrid proteins;

25 Figure 6 depicts construction of plasmids pML105 and pML103 for *in vitro* transcription/translation of LBP/BPI hybrid proteins;

Figure 7 depicts construction of a vector pIC112 encoding a BPI (1-159)/LBP (158-197) hybrid protein;

Figure 8 depicts a graph illustrating the pharmacokinetics of ^{125}I labeled rLBP₂₅ and rBPI₂₃;

Figure 9 depicts binding of rLBP₂₅ to *E. coli* J5 lipid A;

Figure 10 depicts competition by rBPI₂₃ and by rLBP₂₅ for the binding of ^{125}I -rLBP₂₅ to immobilized lipid A;

Figure 11 depicts competition by rBPI₂₃ and by recombinant LBP holoprotein (rLBP) for the binding of ^{125}I -rLBP to immobilized lipid A;

Figure 12 depicts the ability of rLBP₂₅ and rLBP to inhibit the LAL assay;

Figure 13 depicts the effect of rLBP₂₅ and rLBP on binding uptake of ^{125}I -labeled LPS by THP-1 cells;

Figure 14 depicts the effect of rLBP₂₅ and rLBP molecules on TNF production by THP-1 cells;

Figures 15A and 15B depict the effect of rLBP on Tissue Factor (TF) and TNF production, respectively, in PBMCs;

Figure 16 depicts the effect of rLBP on TNF production in PBMCs;

Figure 17 depicts the effect of rLBP₂₅ on TNF production in PBMCs;

Figure 18 depicts the effect of LBP molecules on Tissue Factor production in PBMCs; and

Figure 19 depicts the effect of rLBP₂₅ on LPS induced endothelial adhesiveness for neutrophils;

Figure 20 depicts the effect of rLBP on bacterial binding to monocytes;

Figure 21 depicts the effect of rLBP₂₅ on bacterial binding to monocytes;

Figure 22 depicts the binding of rLBP and rLBP₂₅ in an LBP sandwich ELISA assay; and

5 Figure 23 depicts a homology comparison of the amino-terminal amino acid residues of human LBP and human BPI.

DETAILED DESCRIPTION

The present invention encompasses LBP protein derivatives
10 and LBP derivative hybrid proteins which are characterized by the ability to bind to LPS but which lack the carboxy terminal-associated immunostimulatory element(s) characteristic of the LBP holoprotein and thus lack the CD14-mediated immunostimulatory activity characteristic of LBP holoprotein. Preferred LBP protein derivatives are characterized as
15 including N-terminal LBP fragments having a molecular weight of about 25 kD. Most preferred are LBP N-terminal fragments characterized by the amino acid sequence of the first 197 amino acids of the amino-terminus of LBP set out in Figure 1 and SEQ ID NOS:1 and 2. It is also contemplated that LBP protein derivatives containing N-terminal fragments considerably
20 smaller than 25 kD and comprising substantially fewer than the first 197 amino acids of the N-terminus of the LBP holoprotein molecule are suitable for use according to the invention provided they retain the ability to bind to LPS. Thus, specifically contemplated are LBP derivatives comprising part or all of one or more of three regions (defined by LBP amino acid
25 sequences 17-45, 65-99 and 141-167) corresponding (by reason of amino acid homology) to LPS binding regions (comprising amino acid sequences 17-45, 65-99 and 142-169) of BPI. Moreover, it is contemplated that LBP

protein derivatives comprising greater than the first 197 amino acid residues of the holo-LBP molecule, i.e., including amino acids on the carboxy-terminal side of residue 197 of rLBP as disclosed in Figure 2 and SEQ ID NOS:3 and 4 will likewise prove useful according to the methods of the invention provided they lack CD14-mediated immunostimulatory activity. It is further contemplated that those of skill in the art are capable of making additions, deletions and substitutions of the amino-acid residues of SEQ ID NOS:1-4 without loss of the desired biological activities of the molecules. Such, LBP protein derivatives may be obtained by deletion, substitution, addition or mutation, including mutation by site-directed mutagenesis of the DNA sequence encoding the LBP holoprotein, wherein the LBP protein derivative maintains LPS-binding activity and lacks CD14-mediated immunostimulatory activity. One preferred LBP derivative is that wherein the alanine residue at position 131 of the illustrative LBP (1-197) polypeptide fragment is substituted with a cysteine residue. The resulting LBP (1-197) (Cys 131) polypeptide may have the ability to dimerize via interchain disulfide bond formation through cysteine 131 and the resulting dimer may be characterized by improved biological activity.

Also contemplated are LBP derivative hybrid proteins including LBP/BPI hybrid proteins [but excluding the hybrid designated LBP(1-197)/BPI(200-456) noted above] and LBP-Ig fusion proteins which are characterized by the ability to bind LPS but which lack CD-14 immunostimulatory activity. A preferred LBP/BPI hybrid protein of the invention is a protein comprising one or more portion of the amino-terminal half of LBP, e.g., selected from within amino acid residues 1-197 of LBP, and a one or more portions of the amino-terminal half of BPI, e.g., selected from within amino acid residues 1-199 of BPI.

Other LBP hybrid proteins comprise LBP amino acid sequences into which all or portions of LPS binding domains of other LPS binding proteins (such as BPI) have been inserted or substituted. Preferred LBP hybrid proteins include those in which all or portions of the LPS binding domains of BPI (comprising BPI residues 17-45, 65-99 and 142-169) are substituted into the corresponding region of LBP. Portions of such BPI domains substituted into the hybrid proteins may comprise as few as five continuous amino acids but preferably include ten or more continuous amino acids.

LBP derivative hybrid proteins in which all or portions of the LPS binding regions of BPI are substituted into the corresponding region of LBP thus include those comprising at least a part of an LPS binding domain of BPI selected from the group of amino acid sequences consisting of:

ASQQGTAALQKELKRIKPDYSDSFKIKH (SEQ ID NO:17) designated Domain I comprising the amino acid sequence of human BPI from about position 17 to about position 45;

SSQISMVPNVGLKFSISNANIKISGKWKAQKRFLK (SEQ ID NO:18) designated Domain II comprising the amino acid sequence of human BPI from about position 65 to about 99; and

VHVHISKSKVGWLIQLFHKKIESALRNK (SEQ ID NO:19) designated Domain III comprising the amino acid sequence of human BPI from about position 142 to about position 169.

These LPS binding domains of BPI correspond to LBP regions consisting of:

AAQEGLLALQSELLRITLPDFTGDLRIPH (SEQ IS NO:20) comprising the amino acid sequence of human LBP from about position 17 to about position

45;

HSALRPVPGQGLSLSISDSSIRVQGRWKVRKSFFK (SEQ ID NO:21)
comprising the amino acid sequence of human LBP from about position 65
to about 99; and

5 VEVDMSGDLGWLLNLFHNQIESKFQKV (SEQ ID NO 22) comprising
the amino acid sequence of human LBP from about position 141 to about
position 167.

According to another aspect of the invention, DNA
sequences are provided which encode the above-described LBP protein
10 derivatives and LBP derivative hybrid proteins. Also provided are
autonomously replicating DNA plasmid vectors including such DNA
sequences and host cells stably transformed or transfected with such DNA
sequences in a manner allowing their expression. Transformed host cells
of the invention are of manifest utility in procedures for the large-scale
15 production of the LBP protein derivatives and LBP derivative hybrid
proteins of the invention involving the cultured growth of the hosts in a
suitable medium and the isolation of the proteins from the cells or their
growth medium.

The invention further provides novel pharmaceutical
20 compositions comprising an LBP protein derivative or an LBP derivative
hybrid protein which retains LPS-binding activity and lacks CD14-mediated
immunostimulatory activity together with pharmaceutically acceptable
diluent, adjuvants, and carriers. The compositions are useful in methods
for treating a gram-negative bacterial infection, including the sequelae
25 thereof such as endotoxin related shock, and one or more of conditions
associated with gram-negative bacterial infection and resulting endotoxic
shock such as disseminated intravascular coagulation, anemia,

thrombocytopenia, leukopenia, adult respiratory distress syndrome, renal failure, hypotension, fever and metabolic acidosis. Such methods comprise administering an LBP protein derivative or LBP derivative hybrid protein to a subject suffering from a gram-negative bacterial infection, including
5 the sequelae thereof.

When employed for treatment of a gram-negative bacterial infection, including the sequelae thereof, LBP protein derivatives and LBP derivative hybrid proteins of the invention are preferably administered parenterally and most preferably intravenously in amounts broadly ranging
10 from about 0.1 milligram and about 100 milligrams per kilogram of body weight of the treated subject with preferred treatments ranging from about 1 milligram and 25 milligrams per kilogram of body weight. It is contemplated that administration of LBP derivative protein derivatives, such as rLBP₂₅, and LBP hybrid proteins may be useful as one aspect of a
15 combination therapy in which BPI or other antibiotics are administered to a subject.

According to a further aspect of the invention, LBP protein derivatives and LBP derivative hybrid proteins may be administered in combination with other therapeutic compositions which are not strictly
20 antibiotics but rather which neutralize the endotoxic effects of LPS such as anti-LPS antibodies and antibodies to constituents of the LPS mediated toxic cascade such as anti-TNF antibodies.

The following detailed description relates to the manufacture and properties of LBP protein derivatives and LBP hybrid proteins of the
25 invention. More specifically, Example 1 relates to the construction of vectors for expression of an exemplary LBP protein derivative, rLBP₂₅. Example 2 relates to the construction of vectors for expression of rLBP.

Examples 3 and 4 relate to the incorporation of the vectors of Examples 1 and 2 into appropriate host cells and further describes the expression and purification of rLBP₂₅ and rLBP. Examples 5 and 6 relate to construction of vectors encoding LBP/BPI hybrid proteins. Example 7 relates to *in vitro* transcription translation of the LBP/BPI hybrid protein, LBP(1-43)/BPI(44-199) and BPI(1-159)/LBP(158-197). Example 8 relates to the pharmacokinetics of rLBP₂₅, rLBP and rBPI₂₃ *in vivo*. Example 9 relates to the binding of rLBP₂₅ and rLBP to Lipid A. Example 10 relates to competition by rLBP₂₅ and rBPI₂₃ for the binding of ¹²⁵I-rLBP₂₅ to immobilized lipid A. Example 11 relates to competition by rLBP and rBPI₂₃ for the binding of ¹²⁵I-rLBP to immobilized lipid A. Example 12 relates to the effect of rLBP₂₅ and rLBP on an LAL assay. Example 13 relates to the effect of rLBP₂₅ and rLBP on the binding/uptake ¹²⁵I-labeled LPS on TNF production by a human monocyte cell line THP-1. Example 14 relates to the effect of rLBP₂₅ and rLBP on TF and TNF production by isolated PBMCs. Example 15 relates to the effect of rLBP₂₅ on LPS-induced adhesiveness of endothelial cells for neutrophils. Example 16 relates to the effect of rLBP and rLBP₂₅ on bacterial binding to monocytes and polymorphonuclear cells. Example 17 relates to a sandwich ELISA assay for rLBP and rLBP₂₅. Example 18 relates to construction of vectors for production of LBP (1-197) (Cys 131). Example 19 relates to construction of vectors for production of LBP-IgG hybrid fusion proteins. Example 20 relates to *in vitro* transcription/translations of truncated LBP fragments and determination of their ability to mediate LPS stimulation of TNF activity. Example 21 relates to construction of vectors for production of LBP/BPI hybrid proteins. Example 22 relates to construction of vectors for production of LBP/BPI hybrid proteins comprising BPI/LBP active

domain replacement and partial replacement mutants. Example 23 relates to properties of synthetic LBP peptides.

EXAMPLE 1

5 Construction of Vectors for Expression of rLBP₂₅ Protein

A. Cloning and sequencing of human rLBP₂₅

The DNA encoding amino acids 1-197 of human LBP without the signal sequence (designated "rLBP₂₅") was obtained by PCR using human liver poly (A)⁺ RNA (Clontech Laboratories, Palo Alto, CA) as the source of material for amplification. Reverse transcription of the RNA to cDNA and PCR amplification were carried out using the GeneAmp RNA PCR Kit (Perkin Elmer Cetus, Norwalk, CT) according to the manufacturer's protocols. The sequence of human LBP was obtained from GenBank, accession number M35533, as published by Schumann et al., *Science*, 249:1429-1431 (1990). The 5' PCR primer corresponded to the amino terminal sequence of the coding region of mature LBP and included a *BsmI* recognition site at its 5' end. The sequence of this primer, LBP-Bsm, was: 5'-GAATGCAGCCAACCCCGGCT TGGTCGCCA-3' (SEQ ID NO:5). The 3' PCR primer was designed to place a stop codon and an *XhoI* site following the isoleucine at amino acid position No. 197. The sequence of this primer, LBP-2, was: 5'-CTCGAGCTAAATC TCTGTTGTAAGTGGC-3' (SEQ ID NO:6). This amino acid was chosen as the endpoint of rLBP₂₅ based on sequence homology with an amino-terminal active fragment of BPI (designated rBPI₂₃).

25 The amplified rLBP₂₅ DNA was blunt-end cloned into *SmaI* cut pT7T318U (Pharmacia, LKB Biotechnology, Piscataway, NJ) to generate plasmid pIC106. The LBP insert in pIC106 was sequenced using

Sequenase (USB). The rLBP₂₅ DNA sequence obtained and the derived amino acid sequence is set out in SEQ ID NOS:1 and 2. The sequence differs in two areas from the sequence of the corresponding region of LBP as published by Schumann et al. (1990) *Science*, 249:1429-1431, which
5 involve changes in amino acids. These sequence differences are detailed in Table 1.

B. Construction of Vectors for Expression of rLBP₂₅ in Mammalian Cells

A vector for the expression of rLBP₂₅ using the BPI signal
10 sequence was constructed using the rLBP₂₅ coding region sequence isolated from PIC106 by digestion with *BsmI*, blunt ending with T4 polymerase, and digestion with *XhoI*. This was ligated to two fragments isolated from pING4503 (a plasmid described in co-owned and copending U.S.S.N. 07/885,911 filed May 19, 1992 by Theofan et al. which is hereby
15 incorporated by reference). Briefly, the construction of pING4503 is based on plasmid pING2237N which contains the mouse immunoglobulin heavy chain enhancer element, the LTR enhancer-promoter element from Abelson murine leukemia virus (A-MuLV) DNA, the SV40 19S/16S splice junction at the 5' end of the gene to be expressed, and the human genomic gamma-1
20 polyadenylation site at the 3' end of the gene to be expressed. Plasmid pING2237N also has a mouse dihydrofolate reductase (DHFR) selectable marker. The two fragments from pING4503 were the vector fragment generated by digestion with *ClaI* and *XhoI*, and a fragment containing the BPI signal sequence generated by digestion with *EagI*, blunt ending with
25 T4 polymerase, and digestion with *ClaI*. This ligation resulted in a fusion of the BPI signal to the rLBP₂₅ coding region, keeping the correct reading frame, and generating pING4505 (DHFR gene). The corresponding *gpt*

vector, designated pING4508, was generated by subcloning the *SaII* to *SsrII* insert from pING4505 into the *SaII* and *SsrII* vector fragment from pING3920 (the structure of which is described in co-owned and copending U.S.S.N. 07/718,274 filed June 20, 1991 by Grinna et al., which is hereby incorporated by reference). The construction of pING4505 is outlined in Figure 3.

EXAMPLE 2

Construction of Vectors for Expression of rLBP Proteins

A. Cloning and Sequencing of Human rLBP

The DNA encoding full length human rLBP (amino acids 1-452, designated "rLBP," plus the 25 amino acid signal sequence) was obtained by PCR using human liver poly (A)⁺ RNA (Clontech Laboratories, Palo Alto, CA) as described for rLBP₂₅ above. The 5' PCR primer introduced a *SaII* recognition site at the 5' end of the LBP signal. The sequence of this primer, LBP-3, was: 5'-CATGTCGACACCATGGGGGCCTTG G-3' (SEQ ID NO:7). The 3' PCR primer was designed to introduce an *SsrII* site following the stop codon of LBP. The sequence of this primer, LBP-4, was: 5'-CATGCCGCGGTCAAACCTCTCATGTA-3' (SEQ ID NO:8). The amplified rLBP DNA was blunt-end cloned into *SmaI* cut pT7T318U to generate plasmid pIC128. The LBP insert in pIC128 was sequenced using Sequenase (USB). The rLBP DNA sequence obtained and the derived amino acid sequence are shown in SEQ ID NOS:3 and 4. Additional differences were found between the LBP sequence in pIC128 and the sequence of LBP as published by Schumann et al., *Science* 249:1429-1431 (1990). The rLBP sequence actually encoded 456 amino acids; there was

an insertion of 4 amino acids at position 241. The rLBP sequence in pIC128 additionally contained several nucleotide substitutions that did not change the amino acids compared to Schumann et al. All the differences are highlighted in Table 1. The amino acid sequence is identical to the recently published sequence of LBP β (Seilhamer, PCT International Application WO 93/06228), however, the nucleotide sequence of rLBP herein differs from that of the published LBP β sequence at the sequence encoding amino acids 152 and 179 as shown in Table 1.

5

10

15

20

Table 1 LBP Sequence Differences				
Amino Acid Position 1	rLBP Sequence		Schumann LBP Sequence	
	Nucleotide	Protein	Nucleotide	Protein
-21	GCC	A	GCA	A
72	CCT	P	CCC	P
129	GTT	V	GGT	G
130	ACT	T	TAC	Y
131	GCC	A	TGC	C
132	TCC	S	CTC	L
148	GAC	D	GAT	D
149	TTG	L	TCG	S
152 ²	CTG	L	CTC	L
179	TCG/TCA ³	S	TCA	S
241-245	GTC ATG AGC CTT CCT (SEQ ID NO:9)	VMSLP (SEQ ID NO:10)	GCT	A
411	TTC	F	CTC	L

¹ Amino Acid positions as numbered in SEQ ID NO:4.

² This amino acid position not identified as different for LBP- β .

³ Both nucleotide sequences were found in different PCR-derived clones.

This amino acid position also not identified as different for LBP- β .

B. Construction of Vectors for Expression of rLBP in Mammalian Cells

To construct a vector for expression of rLBP in mammalian cells, pIC128 was digested with *SaII* and *SstII*, the LBP insert was isolated, and then subcloned into *SaII*- and *SstII*-digested vector pING4222 which is described in co-owned and copending U.S.S.N. 08/013,801 filed February 2, 1993 which is hereby incorporated by reference. The resulting rLBP vector was designated pING4539 and also contained the DHFR gene for selection.

EXAMPLE 3

Production and Purification of rLBP

Mammalian cells are preferred host cells for production of LBP protein derivatives of the invention because such cells allow secretion and proper folding of heterodimeric and multimeric proteins and provide post-translational modifications such as pro-sequence processing and glycosylation.

Mammalian cells which may be useful as hosts for the production of LBP protein derivatives (including LBP/BPI hybrid proteins and rLBP-Ig fusion proteins) include cells of lymphoid origin, such as the hybridoma Sp2/O-Ag14 (ATCC CRL 1581) and cells of fibroblast origin, such as Vero cells (ATCC CRL 81), CHO-K1, CHO-DXB11, or CHO-DG44. The latter cell line (a DHFR⁻ mutant of CHO Toronto obtained from Dr. Lawrence Chasin, Columbia University) was maintained in Ham's F12 medium plus 10% fetal bovine serum supplemented with glutamine/penicillin/streptomycin (Irvine Scientific, Irvine, CA).

CHO-DG44 cells were transfected with linearized pING4539 DNA (40 μ g, digested with *PvuI*, phenol-chloroform extracted and ethanol precipitated) using electroporation. Following recovery, the cells were diluted and 1×10^4 cells were plated per 96-well plate well in selective medium consisting of an α MEM medium lacking nucleosides (Irvine Scientific) and supplemented with dialyzed fetal bovine serum (100 ml serum dialyzed using 4L cold 0.15 NaCl using 6000-8000 cutoff for 16 hours at 4°C). Untransfected CHO-DG44 cells are unable to grow in this medium because they possess the DHFR⁻ mutation and were removed during successive feedings with the selective medium. At 1.5-2 weeks, microcolonies consisting of transfected cells were observed.

Clones were analyzed for the presence of LBP-reactive protein in culture by ELISA using Immulon-II 96 well plates (Dynatech). Supernatant samples were added to the plates, incubated 46 hours, 4°C followed by goat anti-LBP antiserum and peroxidase-labeled rabbit anti-goat anti-serum. The 21 most productive positive clones were expanded in selective α MEM medium and then grown on selective medium supplemented with 0.05 μ M methotrexate. The best producing amplified clone was chosen based on ELISA of supernatants as described above and then expanded in α MEM media containing 0.05 μ M methotrexate for growth in roller bottles.

rLBP was produced in CHO-DG44 cells transfected with the plasmid pING-4539. All incubations were performed in a humidified 5% CO₂ incubator maintained at 37°C. Working stock cultures were grown in DME/F-12 with 10% FCS, and were then seeded into 40 2-liter roller bottles (500 mL per bottle) at a density of 9.6×10^4 cells/mL in DME/F-12 with 5% FCS (4.8×10^7 cells/bottle). Four days later, the culture

supernatants from each bottle were removed and then replaced with 500 mL of fresh media (DME/F-12 with 2.5% FCS). Ten mL of an S-Sepharose (Pharmacia) ion exchange resin slurry (50% v/v, sterilized by autoclaving) was then added to each bottle, and incubation was continued. After 2 days, the media containing S-Sepharose was harvested and replaced with fresh media again containing S-Sepharose. This process was repeated one more time, to yield three harvests of S-Sepharose over a four day period. After each harvest, the S-Sepharose resin was allowed to settle for 1 hour, and the spent media was removed by aspiration. The sedimented S-Sepharose was resuspended in fresh media and pooled. The method of purification using S-Sepharose has been described and claimed in co-owned and copending U.S. Patent Application Serial No. 07/855,501 and in co-owned and copending PCT US93/04752 filed May 19, 1993, the disclosures of which are hereby incorporated by reference in their entirety.

All chromatographic resins used in the purification of rLBP and rLBP₂₅ were depyrogenated by immersion in 0.2N NaOH, 1 M NaCl and then rinsed with pyrogen-free water. All buffers and reagents were prepared with bottled, pyrogen-free water for irrigation (Baxter).

The S-Sepharose resin harvested from culture supernatants was washed successively with 5 volumes of cold 20 mM MES, 0.1 M NaCl, pH 6.8 (two times), 20 mM NaAc, 0.1 M NaCl, pH 4.0 (two times), and 20 mM NaAc, 0.4 M NaCl, pH 4.0 (two times). For each of these sedimentations, the resin (in 1 L roller bottles) was allowed to settle at 1 x g in the cold. The resin from each harvest (100-200 mL settled volume) was next packed into individual 5 x 10 cm columns and equilibrated in 20 mM NaAc, 0.4 M NaCl, pH 4.0 at 4°C. Two of the columns were subsequently washed with 20 mM NaAc, 0.7 M NaCl, pH

4.0, until the absorbance at 280 nm approached zero, and were then batch eluted with 20 mM NaAc, 1.0 M NaCl, pH 4.0. Eluted rLBP was identified by SDS-PAGE in 12.5% gels. These gels also showed that some of the rLBP has eluted in the 0.7 M NaCl wash. Consequently, the third
5 batch of resin (about 300 mL) was eluted with a gradient of NaCl (0.4 M to 1.0 M) in 20 mM NaAc, pH 4.0, and 8.5 mL fractions were collected. SDS-PAGE analysis of column fractions indicated that rLBP eluted between fractions 70 and 100. It was estimated that the amount of total protein present in all three eluates was approximately 800 mg.

10 The S-Sepharose fractions containing rLBP from the three harvests were pooled (850 mL), and one-half of the material (425 ml, or about 400 mg rLBP) was purified further. To this material was added 1275 mL of 20 mM NaAc, pH 4.0, such that the final concentration of NaCl would be approximately 0.25 M, and the solution was applied to a
15 second S-Sepharose column (2.5 x 30 cm) equilibrated at 4°C in 20 mM NaAc, 0.4 M NaCl, pH 4.0. The column was eluted with a gradient of NaCl (0.4 to 1.0 M) in 20 mM NaAc, pH 4.0, and column fractions containing rLBP were again identified by SDS-PAGE and pooled. The final volume of pooled material was 133 ml, and it contained about 260 mg
20 of rLBP.

Final purification of rLBP was accomplished by chromatographing the eluate from the second S-Sepharose column on a 345 mL Sephacryl S-100 size exclusion column equilibrated in 20 mM NaCitrate, 0.15 M NaCl, pH 5.0 at 4°C. Five 25 mL aliquots of the S-
25 Sepharose eluate were successively applied to, and eluted from, the column, and 3.7 mL fractions were collected. SDS-PAGE analysis of the effluent fractions indicated that rLBP eluted as a single band, and peak

26

fractions were pooled and stored at 4°C. The peak fractions from all five S-100 runs were then pooled. The final material (about 245 mg) was >99% pure by SDS-PAGE, and exhibited a molecular weight of about 60 kD.

5

EXAMPLE 4

Production and Purification of rLBP₂₅

CHO-DG44 cells were transfected with linearized pING4505 DNA (40 µg, digested with *PvuI*, phenol-chloroform extracted and ethanol precipitated) using the calcium phosphate method of Wigler, *et al.*, *Cell*, 11:223 (1977). Following calcium phosphate treatment, the cells were plated in T75 flasks and transfectants were obtained by growth in selective medium consisting of an αMEM medium lacking nucleosides (Irvine Scientific) and supplemented with dialyzed fetal bovine serum (100 ml serum dialyzed using 4L cold 0.15 NaCl using 6000-8000 cutoff for 16 hours at 4°C). Untransfected CHO-DG44 cells are unable to grow in this medium because they possess the DHFR⁻ mutation and were removed during successive feedings with the selective medium. At 1.5-2 weeks, only microcolonies consisting of transfected cells were observed. The transfected cells were removed from the flasks by trypsinization and subcloned by limiting dilution in 96 well plates.

Subclones were analyzed for the presence of rLBP₂₅ protein in culture supernatants by anti-gamma ELISA using Immulon-II 96 well plates (Dynatech) with LPS as a pre-coat, followed by culture supernatant, goat anti-LBP anti-serum and peroxidase-labeled rabbit anti-goat antiserum. The positive clones were also retested for LBP specific mRNA. The top

25

producing clone was expanded in selective α MEM medium for growth in roller bottles.

rLBP₂₅ was produced in CHO-DG44 cells transfected with the plasmid pING4505 following procedures similar to those described above in Example 3 for rLBP, with minor modifications. After the harvested S-Sepharose was washed in 20 mM NaAc, 0.4 M NaCl, pH 4.0, rLBP₂₅ was batch eluted by the addition of 20 mM NaAc, 1.0 M NaCl, pH 4.0. To the pooled 1.0 M NaCl eluates from the 3 harvests (862 mL) was added 137 mL of 0.5 M MES, such that the final volume was 999 mL, and the final NaCl concentration was about 0.87 M. At this stage, it was estimated that the amount of total protein present was about 775 mg.

A portion of S-Sepharose eluate (549 mL) was diluted with water such that the final volume was 1600 mL, and the final NaCl concentration was about 0.3 M. This diluted material was applied to a 2.5 x 20 cm column of Q-Sepharose previously equilibrated in 20 mM MES, 0.2 M NaCl, pH 5.5 at 4°C. The eluate flowing through the column (which contains the rLBP₂₅) was then applied to a second S-Sepharose column (2.5 x 30 cm), and the column was eluted with a gradient of 0.4 M to 1.2 M NaCl in 20 mM NaAc, pH 4.0. rLBP₂₅-containing fractions were identified by SDS-PAGE and peak fractions were pooled. This pooled material (60 mL) was divided into 20 mL aliquots and sequentially chromatographed on Sephacryl S-100. Peak fractions from the three runs were pooled and rechromatographed over the same S-100 column. The final material (about 33 mg) was >99% pure by SDS-PAGE, and exhibited a molecular weight of about 22 kDa.

EXAMPLE 5**Construction of Vectors for Expression of
LBP (1-43)/BPI (44-199) Hybrid Protein**

5 In this example vectors encoding a hybrid protein [LBP (1-43)/BPI (44-199) hybrid] comprising the first 43 amino-terminal amino acids of LBP and amino acid residues 44-199 of BPI (for BPI see [SEQ ID NOS:11 AND 12] were constructed.

A. Construction of Intermediate Vector pIC111

10 Intermediate vector pIC111 was constructed according to the following procedure as set out in Figure 4. A 250 bp fragment was isolated from pING4505 that encoded the BPI signal sequence and amino acids 1-43 of LBP by digesting with BamHI, blunting the end with T4 polymerase, then cutting with *Sal* I. To generate a fragment of BPI that would provide a blunt junction beginning with amino acid 44, a PCR
15 fragment was produced using two primers. The sequence of primer BPI-18 was 5' AAGCATCTTGGGAAGGGG 3' [SEQ ID NO:13] and the sequence of primer BPI-11 was 5' TATTTTGGTCATTACTGGCAGAGT 3' [SEQ ID NO:14]. The BPI-containing plasmid pING4502 was used as template. Plasmid pING4502 is essentially the same as plasmid pING4503,
20 but does not contain the 30 bp of the 5' UT region of BPI and has the gpt gene instead of DHFR gene for selection. The PCR amplified DNA was digested with *Afl*III and the resulting 100 bp fragment, encoding BPI residues 44 through about 76 was purified. This fragment was ligated
25 together with the 250 bp BPI signal/LBP 1-43 fragment described above into the *Sal*I - *Afl*III vector fragment from pIC110 (BPI in T7T3) to generate pIC111.

B. Construction of Mammalian Expression Vector pING4525

The insert in pIC111 encoding LBP (1-43)/BPI (44-199) with BPI signal was digested with *SaII* and *SsrII* and subcloned into *SaII* - *SsrII* digested pING4502 vector to generate mammalian expression vector pING4525. The construction of pING4525 is set out in Figure 5.

C. Construction of Vector for *In Vitro* Transcription, Translation (pML105)

The construction of *in vitro* transcription plasmid pML105 is set out in Figure 6. pING4525 was digested with *NotI*, treated with mung bean nuclease to blunt the end, then digested with *XhoI*. The resulting 600 bp fragment, which encodes LBP (1-43)/BPI (44-199) without a signal sequence, was gel purified. This fragment was subcloned into pIC127 plasmid which had been digested with *NcoI*, treated with T4 polymerase then *XhoI*, to generate pML105. pIC127 is essentially pGEM1 (Promega, WI) with a BPI₂₃ insert cloned downstream of the SP6 RNA polymerase promoter designed for *in vitro* transcription of the BPI. Digestion of pIC127 with *NcoI* followed by T4 polymerase (to fill in) generates an ATG that can be joined in a blunt junction to a sequence that can then be transcribed in a cell free system.

EXAMPLE 6**Construction of Vectors for Expression of
BPI (1-159)/LBP (158-197) Hybrid Protein**

In this example a vector encoding a hybrid protein [BPI (1-159)/LBP (158-197)] comprising the first 159 amino terminal amino acids of BPI and amino acid residues 158-197 of LBP was constructed.

A. Construction of intermediate pIC112.

Plasmid pING4505 was digested with *EcoRI* and *SstII* and the 135 bp fragment corresponding to LBP 158-197 (plus stop codon) was gel purified. This fragment was ligated with 2 fragments from pIC110, the 250 bp *AflIII* - *EcoRI* fragment and the vector fragment (*SstII* - *AflIII*) to generate pIC112 as set out in Figure 7.

B. Construction of Mammalian Expression Vector pING4526.

The insert encoding BPI (1-159)/LBP (158-197) was excised from pIC112 with *SalI* and *SstII* and subcloned into *SalI* - *SstII* digested pING4502 to generate pING4526 as set out in Figure 5.

C. Construction of Vector for *In Vitro* Transcription/Translation (pML103)

pING4526 was used as the source of a 430 bp *EcoRI*- *SstII* fragment (encoding BPI 61-159/LBP 158-197). This fragment was subcloned into the *SstII* - *EcoRI* vector fragment from pIC127 to generate pML103, encoding the BPI (1-159)/LBP (158-197) insert in a vector for *in vitro* transcription. This construction is set out in Figure 6.

EXAMPLE 7***In Vitro* Transcription/Translation and
Lipid A Binding Activity of LBP/BPI Hybrid Protein**

In this example, *in vitro* transcription/translation reactions were carried out for plasmids encoding BPI₂₃ and the LBP/BPI hybrid proteins of Examples 5 and 6. Specifically, the TNTTM Coupled Reticulocyte Lysate System Kit (Promega, WI) with SP6 RNA polymerase was used to carry out *in vitro* transcription/translation of plasmids pML103 and pML105 to produce hybrid LBP/BPI proteins. The kit is a complete

system, which provides all the reagents necessary to make protein. One adds only the DNA template specifying the protein sequence of interest, a radiolabeled amino acid (^{35}S -methionine) and a ribonuclease inhibitor (Promega's recombinant RNasin).

5 The protocol used to generate the LBP/BPI hybrid proteins was essentially that suggested by Promega's Technical Bulletin #126. Reactions were prepared in volume of 50 μL containing: TNT Rabbit Reticulocyte Lysate, TNT Reaction Buffer, TNT SP6 RNA Polymerase, amino acid mixture minus Methionine, ^{35}S -Methionine used was *in vivo*
10 cell labelling grade purchased from Amersham (catalog #SJ.1015). One microgram of DNA from either pML103 or pML105 was put into the *in vitro* transcription-translation system. As specified by Promega, the transcription-translation reactions were incubated for 2 hours in a water bath at 30°C, then analyzed in various ways to assess the protein products
15 made.

The amounts and molecular weights of the LBP/BPI hybrid proteins were determined by SDS PAGE/Phosphorimager analysis (Molecular Dynamics, CA), TCA precipitation and ELISA. The binding of the LBP/BPI hybrid proteins to immobilized J5 Lipid A was also measured.

20 A BPI sandwich ELISA was carried out using rabbit anti-BPI as the capture antibody on the plate and biotin labeled anti-BPI followed by alkaline phosphatase conjugated to streptavidin as the detection system.

A J5 Lipid A binding (RIA) binding assay was carried out using pop-apart Immulon-2 strips coated overnight with 25 ng per well of
25 J5 Lipid A. Two sets of plates (coated and uncoated) were blocked for three hours at 37°C with 0.1% BSA/PBS, washed with PBS/0.05% Tween, then binding was done at 37°C for one hour with 50 μL of a 1:25 dilution

of the transcription-translation reactions. After binding, plates were washed, then placed in scintillation vials with 2 mL of scintillation fluid and counted in a (Beckman) beta-counter.

Comparable amounts of LBP/BPI hybrid protein of the anticipated molecular weight were made for each of the analogs. However, when the total protein in each reaction was quantitated by ELISA, the yields appeared to differ. The absolute numbers obtained in the ELISA may reflect altered epitopes in the LBP/BPI hybrid proteins which may not be recognized equally by the rabbit anti-BPI antibody. In particular, the low amount of pML105 detected by ELISA is thought to underestimate the actual protein yield. The LBP/BPI hybrid proteins bound immobilized J5 Lipid A as well as BPI₂₃ (1-199) made with pIC127. Specific binding (background corrected) for the LBP/BPI hybrid proteins is shown in Table 2. LBP/BPI hybrid proteins of the invention may have such desired properties as LPS-binding activity comparable to rBPI₂₃, while lacking CD14-mediated immunostimulatory activity characteristic of the holo-LBP proteins, and may have advantageous pharmacokinetic properties such as increased half-life as compared with rBPI₂₃ as measured in Example 8 below.

TABLE 2

Plasmid	Description	Yield ELISA	Specific Binding RIA, J5 Lipid A
pIC127	BPI23 (1-199)	57 ng	140,000 cpm
pML103	BPI (1-159) LBP (158-197)	100 ng	210,000 cpm
pML105	LBP (1-43) BPI (44-199)	12 ng	150,000 cpm

EXAMPLE 8**Pharmacokinetics of rLBP₂₅, rLBP
and rBPI₂₃ in Male CD Rats**

(The pharmacokinetics of rLBP₂₅, rLBP and rBPI₂₃ were investigated in rats using an ELISA assay.) The pharmacokinetics of 1 mg/kg rLBP₂₅ was investigated in 3 male CD rats. Blood samples were collected at selected times (from 0.5 minutes to 72 hours) after administration of dose. Plasma samples were then assayed by ELISA using affinity purified rabbit anti-LBP₂₅ as the capture antibody and biotin labelled affinity purified rabbit anti-LBP₂₅ as the secondary antibody.

The plasma concentration-time profile of rLBP₂₅ could be fit to a three-compartment model, with α half-life of 2.3 ± 0.3 minutes, β half-life of 10.8 ± 1.0 minutes, and γ half-life of 101 ± 14 minutes, with a systemic mean residence time of about 9.2 ± 0.8 minutes (Table 3). The rate of clearance of rLBP₂₅ from the plasma was about 10 times faster than

that observed for rLBP, and about 5 times slower than the clearance rate of rBPI₂₃. Unlike BPI, removing the carboxy terminal end of LBP significantly increased its clearance rate. However, the steady state volume of distribution of LBP₂₅ is similar to that of LBP, suggesting that the
5 distribution of LBP was not affected by this modification.

The pharmacokinetics of 1 mg/kg rLBP was investigated in 3 male CD rats. Blood samples were collected at selected times (from 0.5 minutes to 75 hours) after administration of dose. Plasma samples were then assayed by ELISA using affinity purified rabbit anti-LBP₂₅ as the
10 capture antibody and biotin labelled affinity purified rabbit anti-LBP₂₅ as the secondary antibody.

The plasma concentration-time profile of rLBP could be fit to a three-compartment model, with α half-life of 14 ± 2 minutes, β half-life of 130 ± 16 minutes, γ half-life of 561 ± 37 minutes (Table 3). The
15 rate of clearance of rLBP from the plasma was very slow, about 60 times slower than rBPI₂₃.

Table 3						
Pharmacokinetics Parameter Values from the Serum Clearance Curve of 1 mg/kg rBPI ₂₃ , rLBP ₂₅ and rLBP						
(mean \pm se)						
Test Article	V _c mL/kg	V _{ss} mL/kg	Clearance mL/min/kg	MRT minutes	t _{1/2} α minutes	t _{1/2} β minutes
rBPI ₂₃	62.3 \pm 15	78 \pm 16	25.5 \pm 5.4	3.0 \pm 0.0	1.62 \pm 0.06	18.0 \pm 3.0
rLBP ₂₅	46.8 \pm 0.6	269 \pm 60	5.1 \pm 0.4	51.8 \pm 8.9	2.33 \pm 0.3	10.8 \pm 1.0
rLBP	55.7 \pm 9.0	208 \pm 77	0.45 \pm 0.1	437.6 \pm 55	13.57 \pm 2.0	129.7 \pm 16

V_c = volume of distribution of the central compartment.

V_{ss} = steady state volume of distribution.

MRT = total body mean residence time.

t_{1/2} α = alpha half-life.

t_{1/2} β = beta half-life.

EXAMPLE 9

Binding of rLBP₂₅ and rLBP to Lipid A

In this example, the binding of rLBP₂₅ to immobilized lipid A was determined according to the method of Gazzano-Santoro et al., *Infect. Immun.* 60: 4754-4761 (1992) but utilizing ¹²⁵I labeled rLBP₂₅ instead of ¹²⁵I labeled rBPI₂₃. Radioiodination of proteins was performed essentially as previously described (Gazzano-Santoro et al.) except that the iodination was performed in the absence of Tween 20 and the iodinated protein was exchanged by gel filtration into 20 mM citrate, pH 5.0, 0.15M NaCl, 0.1% F68 (Poloxamer 188) 0.002% Polysorbate 80.

Specifically, an *E. coli* J5 lipid A suspension was sonicated, diluted in methanol to a concentration of 0.1 µg/mL and 100 µL aliquots were allowed to evaporate in wells (Immulon 2 Removawell Strips, Dynatech) at 37°C overnight. The wells were then blocked with 215 µL of D-PBS/0.1% BSA (D-PBS/BSA) for three hours at 37°C. The blocking buffer was discarded, the wells were washed in D-PBS/0.05% Tween 20 (D-PBS/T) and incubated overnight at 4°C with D-PBS/T containing increasing amounts of ¹²⁵I labeled rLBP₂₅, or rBPI₂₃ as described in Gazzano-Santoro et al. After three washes, bound radioactivity was counted in a gamma counter. The binding to wells treated with (D-PBS/BSA) only was taken to represent nonspecific binding; specific binding was defined as the difference between total and nonspecific binding. For rLBP, the experiment was performed as described above except that the wells were blocked in D-PBS/1%BSA, washed with D-PBS/0.1%BSA and the binding incubation buffer was D-PBS/0.1% BSA. The resulting data were fitted to a standard binding equation by computerized non-linear curve

fitting (Leatherbarrow, *Trends Biochemical Sciences*, 15: 455-458 (1990); GraFit Version 2.0, Erathicus Software Ltd., Staines, UK). According to these experiments the labeled rLBP₂₅ had a K_d of about 76 nM (Figure 9) and the rLBP had a K_d of about 60 nM, while rBPI₂₃ had a K_d of approximately 3 nM, indicating an approximately 15-25 fold lower binding affinity of rLBP₂₅ and rLBP, as compared with rBPI₂₃, for LPS. However, the binding of rLBP₂₅ and rLBP were comparable, demonstrating that the LPS-binding site of rLBP is localized in the N-terminal portion of the molecule.

EXAMPLE 10

Competition by rBPI₂₃ and by rLBP₂₅ for the Binding of ¹²⁵I-rLBP₂₅ to Immobilized Lipid A

In this example, the inhibition of ¹²⁵I-labeled rLBP₂₅ binding to *E. coli* J5 lipid A by unlabeled rBPI₂₃ or rLBP₂₅ was determined. Specifically, *E. coli* J5 lipid A was diluted in methanol to a concentration of 1 µg/mL and 50 µL aliquots were allowed to evaporate in wells overnight at 37°C. The wells were then blocked in D-PBS/BSA. Increasing amounts of unlabeled rBPI₂₃ or rLBP₂₅ in a 50 µL volume were then added and the wells were incubated overnight at 4°C in D-PBS/T. Twenty microliters of ¹²⁵I-rLBP₂₅ (0.65 x 10⁶ cpm, specific activity 5.29 µCi/µg) were added directly to each well and further incubated for one hour at 4°C. After three washes in D-PBS/T, wells were counted. Figure 10 shows that although rLBP₂₅ is able to compete with ¹²⁵I-rLBP₂₅ for binding to lipid A, rBPI₂₃ is a better competitor. These results are consistent with the difference in relative affinity as shown in Example 9.

EXAMPLE 11**Competition by rBPI₂₃ and by rLBP for the Binding
of ¹²⁵I-rLBP to Immobilized Lipid A**

In this example, the inhibition of ¹²⁵I-labeled rLBP binding
5 to *E. coli* J5 lipid A by rBPI₂₃ and rLBP was determined. Specifically, *E. coli* J5 lipid A was diluted in methanol to a concentration of 1 µg/mL and 100 µL aliquots were allowed to evaporate in wells overnight at 37°C. Following incubation, the wells were blocked in D-PBS/1%BSA. Increasing amounts of unlabeled rBPI₂₃ or rLBP mixed with a fixed
10 amount of ¹²⁵I-rLBP (1.45 x 10⁶ cpm, specific activity 1.74 µCi/µg) were added to wells in a 100µL volume and incubated overnight at 4°C in D-PBS/BSA. After three washes in D-PBS/BSA, wells were counted. Figure 11 shows the results wherein increasing amounts of both rLBP and rBPI₂₃ compete with radiolabeled rLBP binding for the immobilized lipid A.
15 These results show that rBPI₂₃ was more potent than rLBP at competing for rLBP (Figure 11) binding to lipid A consistent with the difference in relative affinity as shown in Example 9.

EXAMPLE 12**20 Ability of rLBP₂₅ and rLBP to Inhibit the LAL Assay**

In this example, the effect of rLBP₂₅ and rLBP were compared for their ability to inhibit the *Limulus* ameocyte lysate (LAL) assay. Specifically, increasing concentrations of the LPS binding proteins were incubated in the presence of 2 ng/mL of *E. coli* 0113 LPS (60 µL
25 volume) for three hours at 37°C. The samples were then diluted with PBS to bring the LPS concentration to 333 pg/mL and the amount of LPS activity was determined in the chromogenic LAL assay (Whittaker

Bioproducts, Inc.). The results shown in Figure 12 show that rLBP₂₅ and the rLBP protein products had comparable endotoxin neutralizing activity in the LAL assay.

5

EXAMPLE 13

Effect of LBP Molecules on Binding/Uptake of ¹²⁵I-labeled LPS and on TNF Production by THP-1 Cells

In this example, the effect of rLBP₂₅ and rLBP on binding/uptake of LPS and TNF production by a human monocyte cell line bearing CD14 receptors on its cell surface was determined. Specifically, THP-1 human monocyte cells (obtained from the American Type Culture Collection Tumor Immunology Bank, 12301 Parklawn Dr., Rockville, MD 20852) were grown to a density of 3.5×10^5 /mL in RPMI 1640 media (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone Laboratories, Logan, UT), 1mM glutamine, 1 mM pyruvate (Gibco), and 10U/mL penicillin and streptomycin (Gibco). The cells were transferred at the same density into the above medium supplemented with 100nM 1,25 dihydroxyvitamin D₃ (Biomol Research Laboratories, Plymouth Meeting, PA) and allowed to grow for another 3 days before use. Tubes containing 2×10^6 cells in 1 mL of RPMI/0.1% BSA were incubated in the presence of 20 ng/mL of ¹²⁵I-labeled Ra LPS (List Biological Laboratories, Campbell CA) with various concentrations of rLBP₂₅, or rLBP. Nonspecific uptake was determined by adding a 2000-fold excess of unlabeled RaLPS for one hour at 37°C. At the end of the incubation time, the cells were removed from the binding medium by centrifugation through a layer of dibutyl/dioctyl

10

15

20

25

phthalate oil and counted. Supernatants were sampled at the end of the assay to determine TNF production.

The presence of rLBP stimulated both the uptake of ^{125}I -labeled LPS (Figure 13) and the release of TNF (Figure 14) by the THP-1 cells. In contrast, if the cells are incubated with rLBP₂₅, there was no significant uptake of ^{125}I -labeled LPS (Figure 13) and no significant TNF production (Figure 14).

EXAMPLE 14

Effect of LBP Molecules on TF and TNF Production in PBMCs

In this example, peripheral blood mononuclear cells (PBMCs) isolated from healthy human donors were used in a model system to determine the effects of rLBP₂₅ and rLBP on LPS-mediated effects. PBMCs include approximately 20% monocytes which are known to produce tissue factor (TF) which is a cell surface protein responsible for initiating blood clotting and tumor necrosis factor (TNF) when stimulated by LPS. Tissue Factor if assembled with factor VII initiates the blood coagulation cascades. TF activity induced by LPS has been implicated in the pathogenesis of disseminated intravascular coagulation (DIC), which is a major complication of gram-negative sepsis. Bone, *Annals Int. Med.* 115:457 (1991).

According to the experimental protocol, PBMCs were prepared from buffy coat from the blood of healthy human donors by separation over Ficoll-Hypaque. The percentage of monocytes (15-25%) in different PBMC preparations was determined by fluorescence activated cell sorting (FACS) analysis based on the expression of CD14 antigen. The

PBMCs (2×10^6 /mL) were incubated in 1 mL Dulbecco's modified Eagle medium containing 25 mM HEPES, at 37°C, 5% CO₂ for 4 hours.

During this incubation, LPS in varying concentrations is present with varying concentrations of rLBP or rLBP₂₅ or 20% fetal bovine serum

5 (FBS). After incubation the cells and media were separated by centrifugation. The cells were washed with Tris-NaCl buffer (0.1 M Tris, 0.15M NaCl, 0.1% bovine serum albumin, pH7.4) then lysed in the same buffer by adding 15mM octyl-beta-D-glycopyranoside and incubated at 37°C for 30 minutes.

10 To determine TNF production by the PBMCs incubated as described above, the supernatant of the PBMCs was assayed for TNF by ELISA (T Cell Sciences, Cambridge, MA).

To determine TF production, total cellular TF activity was determined in the PBMC lysate using a two-stage amidolytic assay, similar
15 to that described by Moore et al., *J. Clin. Invest.* 79:124-130 (1987). In the first stage, the PBMC lysate was mixed with coagulation factor VII and factor X, in order to combine TF with factor VII, which in turn, activated factor X. In the second stage the activity of factor Xa was measured using a chromogenic peptide substrate. Factor VII, factor X and this synthetic
20 peptide substrate (Spectrozyme FXa [MeO-CO-D-CHG-Gly-Arg-pNA]) were the products of American Diagnostica, Greenwich, CT. TF activity was calculated by reference to standard curves obtained with dilutions of rabbit brain thromboplastin (Sigma Chemical Co., St. Louis, MO) in the amidolytic assay.

25 For this assay in a 96 well microtiter plate, cell lysate (containing 10^3 - 10^4 monocytes) or different dilutions of the thromboplastin standard (corresponding to 0.1 to 1 µl buffer (0.1 M Tris, 0.15 M NaCl,

pH 7.4) containing 7 mM CaCl_2 . The mixture was incubated at room temperature for 20 min. Then 50 μl of Spectrozyme FXa, (synthetic substrate for factor Xa; 1.4 mg/ml) was added and the rate of absorbance increase at 405 nm was measured using a kinetic plate reader (Molecular Devices, Menlo Park, CA).

According to one experiment, varying amounts of recombinant rLBP (produced according to the method of Example 3) and 100 or 1000 pg/ml of *E. coli* LPS were contacted with the isolated PBMCs to determine the effect of rLBP on the appearance of TF in the cell lysate and on TNF release. The results in Figures 15a and 15b show that rLBP greatly stimulates the LPS-mediated synthesis of TF and TNF. Similarly, incubation of LPS (100 or 1000 pg/ml) and 20% fetal bovine serum (FBS) also resulted in TF and TNF synthesis. Since LBP is present in serum, the bar-graphs at the right of Figures 15a and 15b show that serum also mediates LPS mediated responses of TF and TNF production. To determine whether the effects of LPS are mediated by the CD14 antigen present on the surface of monocytes, antibodies to this determinant were included in the incubation mixture in other experiments. Addition of anti-CD14 monoclonal antibody (MY4; Coulter Immunology, Hialeah, FL) in the presence of serum or rLBP inhibited the induction of both tissue factor activity and TNF release. The extent of inhibition diminished at higher LPS concentrations.

The use of the PBMC model to determine TF and TNF production was then repeated to compare the effect of rLBP₂₅ with that of recombinant rLBP on LPS-induced TF and TNF production. The results with varying concentrations of LPS shown in Figure 16 compare administration of 20% FBS with serum-free media containing various

concentrations of recombinant rLBP. These results again demonstrate that rLBP potentiates TNF release. The results shown in Figure 17 with varying concentrations of LPS compare administration of 20% FBS with varying concentrations of rLBP₂₅ in serum-free media. In contrast to the results with rLBP, these results demonstrate that rLBP₂₅ does not have an immunostimulatory effect and therefore does not potentiate the release of TNF.

Although rLBP₂₅ could not mediate the CD14-dependent LPS stimulation of monocytes to produce TF or TNF, the ability of rLBP₂₅ to compete with rLBP and inhibit the LPS-mediated appearance of TF on the PBMC cell surface was tested. Specifically, rLBP₂₅ produced according to the method of Example 4 was added in concentrations ranging from 0.1 to 10.0 µg/mL to PMBCs that were incubated with a constant amount of rLBP (2 µg/ml) and stimulated with either 20 pg/mL or 200 pg/mL LPS. The results shown in Figure 18 demonstrate that not only does rLBP₂₅ not mediate LPS-stimulated TF production similar to rLBP but also it effectively competes with rLBP to substantially reduce the LPS-induced TF production. Similar effects were obtained for TNF production.

EXAMPLE 15

Effect of rLBP₂₅ on LPS Induction of Endothelial Cell Adhesiveness for Neutrophils

In this example, the effect of rLBP₂₅ on LPS induction of endothelial cell adhesiveness for neutrophils was studied in an *in vitro* adherence assay. Human umbilical cord endothelial cells (HUVEC) were cultured in a 48-well plate and incubated for 4 hours at 37°C with 10

ng/mL LPS (*E. coli* 0113) in M199 supplemented with 2% FCS, or with the medium only. rLBP₂₅ was added to the LPS-containing wells at concentrations varying from 0.1 to about 100 µg/mL to determine its ability to neutralize the LPS-induced increase in the adhesiveness of endothelial cells. Following the four hour incubation, HUVEC monolayers were washed three times, and 2.5×10^5 ⁵¹Cr-labeled human blood neutrophils in 0.2 mL RPMI-2% FCS were added to each well. The plate was incubated at 37°C for 30 minutes. After the incubation, the supernatant was aspirated and each well was washed with 1 mL of warm medium to remove non-adherent neutrophils. The cells remaining in each well were solubilized with 0.2 mL of 0.25N NaOH and the lysates were counted in a gamma counter. Percent neutrophil adherence was calculated as 100 x cpm lysate/cpm added. The data presented in Figure 19 on the mean of percent neutrophil adherence of four replicates per group. The results shown in Figure 19 demonstrate that rLBP₂₅ at 100 µg/ml completely inhibits the LPS-induced neutrophil adhesion. Under the conditions of this assay, 10 or 1 µg/mL of rLBP₂₅ inhibits 71% and 51%, respectively, of the LPS-stimulated adherence, while 0.1 µg/mL of rLBP₂₅ had no significant inhibitory effect.

20

EXAMPLE 16

Effect of rLBP and rLBP₂₅ on Bacteria Binding to Monocytes

In this example, the effect of rLBP₂₅ on bacterial binding to monocytes and polymorphonuclear neutrophils (PMNs) was compared with that of rLBP. White blood cells (WBC) were isolated by lysis with NH₄Cl of acid-citrate-dextran treated blood and red blood cell ghosts removed by washing. Bacteria were cultured overnight in Trypticase soy broth, washed

25

extensively, incubated for 1 hour at room temperature with 1 mg/ml fluorescein isothiocyanate (FITC), and washed. Most strains were then incubated for 30 minutes at 60°C and washed again. The concentration of bacterial cells was then determined turbidometrically.

5 In order to measure bacterial binding and uptake by WBC, WBC adjusted to 10^7 /ml in pooled normal human serum (NHS) were mixed with a 5-fold excess of bacteria in NHS and incubated. Experiments were terminated by transferring the tubes to 4°C and/or adding cytochalasin B. Cells were then washed extensively with ice cold DPBS
10 and analyzed immediately on a FACScan (Becton Dickinson, San Jose, CA). Binding is defined as the percent of cells with bacteria at time zero. Uptake is defined as the percent of cells with bacteria after 15 minutes at 37°C minus the percent of cells with bacteria at time zero.

For the determination of the effect of rLBP and rLBP₂₅ on
15 bacterial binding to monocytes, isolated WBC in 100% allogeneic human serum mixed with *E. coli* J5 bacteria that had been pretreated with various concentrations of rLBP or rLBP₂₅. As shown in Figures 20 and 21, there was minimal binding (<10%) to either monocytes or PMNs by the bacteria in the absence of rLBP or rLBP₂₅. rLBP mediated a concentration-
20 dependent binding of bacteria to monocytes, that reached 30% at 100 µg/mL rLBP (Figure 20). This rLBP-enhanced binding of bacteria to monocytes was shown to be inhibited by an anti-CD14 monoclonal antibody (mAb MY4) but not by an isotype-matched control (mAb 5C2). In striking contrast, rLBP₂₅ was unable to mediate such a CD14-dependent enhanced
25 binding to monocytes as induced by rLBP (Figure 21). No comparable increase in binding to PMNs is mediated by either rLBP or rLBP₂₅. This

finding is consistent with the much lower levels of CD14 on PMNs as opposed to monocytes.

EXAMPLE 17

LBP Sandwich ELISA

An LBP sandwich ELISA has been developed which utilizes a rabbit anti-rLBP antibody on solid-phase with a biotin-labeled rabbit anti-rLBP as the detector. Both rLBP and rLBP₂₅ were detected by this ELISA (Figure 22). The linear range of the rLBP standard curve was 40 to 800 pg/mL.

In this assay, rBPI produced a signal which was approximately 5 orders of magnitude lower than that of rLBP. For example, the signal produced by 100,000 ng/mL of rBPI was equivalent to the signal produced by 0.7 ng/mL of rLBP. Therefore, endogenous BPI will have a negligible effect on the accurate measurement of LBP.

Fifty microliters of affinity purified rabbit anti-rLBP antibody (1 µg/mL in PBS) were incubated overnight at 2-8°C (or alternatively, 1 hour at 37°C) in the wells of Immulon 2 (Dynatech Laboratories Inc., Chantilly, VA) microtiter plates. The antibody solution was removed and 200 µL of 1% non-fat milk in PBS (blocking agent) was added to all wells. After blocking the plates for one hour at room temperature, the wells were washed three times with 300 µL of wash buffer (PBS/0.05% Tween-20). Standards, samples and controls were diluted in triplicate with PBS containing 1% bovine serum albumin, 0.05% Tween 20 (PBS-BSA/Tween) and 10 units/mL of sodium heparin (Sigma Chemical Co., St. Louis, MO) in separate 96-well plates. rLBP or rLBP₂₅ standard solutions were prepared as serial two-fold dilutions from 100 to 0.012

ng/mL. Each replicate and dilution of the standards, samples and controls (50 μ L) was transferred to the blocked microtiter plates and incubated for one hour at 37°C. After the primary incubation, the wells were washed three times with wash buffer. Biotin-labeled rabbit anti-rLBP antibody was
5 diluted 1/4000 in PBS-BSA/Tween and 50 μ L was added to all wells. The plates were then incubated for one hour at 37°C. Subsequently, all wells were washed 3 times with wash buffer. Alkaline phosphatase-labeled streptavidin (Zymed Laboratories Inc., San Francisco, CA) was diluted 1/2000 in PBS-BSA/Tween and 50 μ L was added to all wells. After
10 incubation for 15 minutes at 37°C, all wells were washed three times with wash buffer and 3 times with deionized water and the substrate p-nitrophenylphosphate (1 mg/mL in 10% diethanolamine buffer) was added in a volume of 50 μ L to all wells. Color development was allowed to proceed for one hour at room temperature, after which 50 μ L of 1 N
15 NaOH was added to stop the reaction. The absorbance at 405 nm was determined for all wells using a Vmax Plate Reader (Molecular Devices Corp., Menlo Park, CA).

The mean absorbance at 405 nm (A_{405}) for all samples and standards (in triplicate) were corrected for background by subtracting the
20 mean A_{405} of wells receiving only sample dilute buffer (no BPI) in the primary incubation step. A standard curve was then plotted as A_{405} versus ng/ml of rLBP or rLBP₂₅. The linear range was selected, a linear regression analysis was performed and concentrations were determined for samples and controls by interpolation from the standard curve.

EXAMPLE 18**Construction of Vectors for Expression of LBP Derivatives**

An LBP derivative was constructed in which the alanine residue at position 131 was mutated to cysteine. This position is analogous to cysteine 132 in the BPI sequence, which appears to be the cysteine involved in homodimer formation. By placing a cysteine in the same position in the LBP sequence, the expressed protein may also have the ability to dimerize via interchain disulfide bond formation through cysteine 131, and the resultant dimer may have increased biological potency as is observed for the BPI dimer. To construct the LBP (1-197) (Cys131) derivative, two complementary oligonucleotides containing the desired mutation were used to replace a portion of the LBP sequence between *BpmI* and *PstI* restriction sites, corresponding to the amino acid sequence from residue 127 to residue 133. These oligonucleotides were LBP15: 5'-CCCACAGTCACGTGCTCCAGCTGCA-3'[SEQ ID NO:15], and LBP16: 5'-GCTGGAGCACGTGACTGTGGGCC-3'[SEQ ID NO:16]. These 2 annealed oligonucleotides were ligated to two fragments from pML116: the -409 bp *HindIII-BpmI* fragment and the -3033 bp *PstI-HindIII* fragment, to generate plasmid pML136. Plasmid pML116 is a vector for use in an *in vitro* transcription/translation system encoding LBP (1-197) which corresponds to mammalian vectors pING4505 and pING4508 described above. Plasmid pML136 is then expressed in an *in vitro* transcription/translation system as described above to produce LBP (1-197) (Cys 131). A corresponding vector for expression in mammalian cells can be constructed according to the methods described previously.

EXAMPLE 19**Vectors for Expression of LBP-IgG Hybrid Fusion Proteins**

Portions of the LBP protein can be included in fusions with the Fc region of human IgG₁ according to the general methods of co-owned and copending U.S. Patent Application Serial No. 08/064,693 filed May 19, 1993, the disclosure of which is hereby incorporated by reference, which teaches the construction of BPI-IgG fusion proteins. These LBP hybrid proteins may include truncated forms of LBP, such as 1-197, and may also include LBP derivatives containing the alanine 131 to cysteine mutation. These LBP hybrid proteins may be expected to form dimers upon recombinant production and may also have improved pharmacokinetics.

EXAMPLE 20***In Vitro* Transcription/Translations of Truncated LBP Fragments and Determination of Their Ability to Mediate LPS Stimulation of TNF Activity**

While rLBP₂₅ and rLBP interact similarly with lipid A/LPS, rLBP and not rLBP₂₅ can stimulate the uptake of LPS and release the TNF by THP-1 cells. These data suggest that the region of LBP responsible for mediation of LPS activity via the CD14 receptor lies between positions 197 and 456 (the end) of the LBP protein sequence. To define this region more precisely, plasmid pML130 containing the entire 456 amino acid sequence of LBP (including silent mutations to introduce a *Cla*I restriction site at position 197-198), was constructed for *in vitro* transcription/translation experiments. Plasmid pML130 corresponds generally to mammalian vector pING4539 described previously. The LBP insert in pML130 is under the

control of the T7 RNA polymerase promoter, which efficiently transcribes RNA from linear DNA templates. Therefore, deletions of the LBP sequence generated via restriction digests of plasmid pML130 in the region between residues 197 and 456 as set out in Table 5 below are used as templates in the *in vitro* transcription/translation reaction to generate truncated LBP protein fragments. The biological activity of these LBP protein fragments are then tested to determine the region of the LBP sequence necessary for mediating the interaction of LPS with the CD14 receptor with the LBP (1-456) holoprotein acting as a positive control for CD14 activity and the rLBP₂₅ (1-197) fragment acting as a negative control. Mammalian vectors for expression of the LBP derivatives are produced and the derivatives are expressed according to the methods disclosed previously.

TABLE 5

Restriction enzyme digest	LBP fragment size (amino acids)
<i>HindIII</i>	456
<i>Eco47III</i>	400
<i>BsiHKAI</i>	342
<i>Bam1105I</i>	298
<i>Bsu36I</i>	244
<i>ClaI</i>	196

EXAMPLE 21**Construction of Vectors for Expression of LBP Hybrid Proteins**

A number of vectors were constructed to express LBP hybrid proteins containing both BPI and LBP sequences derived from the amino-terminal domains of each protein. Such proteins are expected to retain LPS binding activity since the LPS binding domain exists within the amino-terminal region of both BPI and LBP. It is further expected that the hybrid proteins comprising amino terminal portions of both LBP and BPI would be unable to mediate LPS effects via interaction with CD14 receptor. Such proteins may further have advantageous biologic or pharmacokinetic properties. Vectors constructed as intermediates or for use in the *in vitro* transcription/translation system have the designation "pIC" or "pML" followed by a number while vectors intended for expression in mammalian cells have the designation "pING" followed by a number.

A vector encoding an LBP hybrid protein combining the elements of pML105 [LBP (1-43)/BPI (44-199)] and pML103 [BPI (1-159)/LBP (158-197)], namely pML134, was constructed to encode LBP (1-43)/BPI (44-159)/LBP (158-197). The construction of pML134 was accomplished by ligating the -458 bp *NheI-EcoRI* fragment of pML105 to the -3011 bp *EcoRI-NheI* fragment of pML103. In addition to pING4526 already described, another vector for expression of BPI(1-159)/LBP(158-197) in mammalian cells was constructed which contained optimized elements including Kozak initiation sequence, human kappa poly A/Mouse Kappa genomic transcription termination and CMV promoter as described in coowned and co-pending U.S. Application Serial No. 08/013,801 filed February 2, 1993 the disclosure of which is hereby incorporated by reference. The mammalian vector was designated pING4168.

Two other BPI/LBP hybrids, pML117 and pML118, were constructed by taking advantage of common *Pst*I restriction sites contained in the coding region sequence of both proteins. The starting plasmids for these constructs were pIC127, which contains an insert encoding BPI (1-199), and pIC106, which contains an insert encoding LBP (1-197).
pML117, containing an insert encoding BPI (1-137)/LBP (137-197), was constructed by replacing the -200 bp *Pst*I-*Xho*I fragment of BPI in pIC127 with the corresponding LBP fragment from pIC106. pML118, encoding BPI (1-25) LBP (26-135) BPI (137-199) was constructed by replacing an -600 bp *Pst*I-*Pst*I fragment within the BPI coding region of pIC127 with the corresponding LBP fragment from pIC106. The resulting plasmids are used as templates in the *in vitro* transcription/translation reaction method according to Example 7 in order to generate LBP hybrid proteins. Constructs pML103, pML105, pML116, pML117, pML118, pML134 were expressed in the transcription/translation system and the products were all detected by ELISA using appropriate reagents. All the products bound well to Lipid A with the exception of BPI (1-25)/LBP(26-134)/BPI(135-199) which bound poorly. Three products exhibited superior Lipid A binding activity. They were BPI(1-159)/LBP(158-197), BPI(1-135)/LBP(135-197), and LBP(1-43)/BPI(44-159)/LBP(158-197).

Mammalian vectors for expression of the LBP hybrid proteins are produced and the derivatives are expressed according to the methods disclosed previously.

EXAMPLE 22**Construction of Vectors for Expression of LBP Hybrid Proteins
Comprising BPI/LBP Active Domain Replacement Mutants**

rBPI₂₃ possesses three biological activities, LPS binding, heparin binding and bactericidal activity, which have been localized to specific domains of the protein defined by synthetic peptides as disclosed by co-owned and copending U.S. Patent Application Serial No. 08/209,762 filed March 11, 1994 which is a continuation-in-part of U.S. Patent Application No. 08/183,222 filed January 14, 1994, which is a continuation-in-part of U.S. Patent Application No. 08/093,292 filed July 15, 1993 which is a continuation-in-part of U.S. Patent Application Serial No. 08/030,644 filed March 12, 1993 the disclosures of which are incorporated herein by reference. Domain I of BPI includes the amino acid sequence of human BPI from about position 17 to about position 45 having the sequence: BPI Domain I ASQQGTAALQKELKRIKPDYSDSFKIKH (SEQ ID NO:17); Domain II of BPI includes the amino acid sequence of human BPI from about position 65 to about position 99 having the sequence BPI Domain II SSQISMVPNVGLKFSISNANIKISGKWKAQKRFLK (SEQ ID NO:18) while Domain III of BPI includes the amino acid sequence of human BPI from about position 142 to about position 169 having the sequence BPI Domain III VHVHISKSKVGWLIQLFHKKIESALRNK (SEQ ID NO:19) The corresponding domain I of LBP includes the amino acid sequence of human LBP from about position 17 to about position 45 having the sequence LBP Domain I AAQEGLLALQSELLRITLPDFTGDLRIPH (SEQ IS NO:20); Domain II of LBP includes the amino acid sequence of human LBP from about position 65 to about position 99 having the sequence LBP Domain II

HSALRPVPGQGLSLSISDSSIRVQGRWKVRKSFFK (SEQ ID NO:21);
 while Domain III of LBP includes the amino acid sequence of human LBP
 from about position 141 to about position 167 having the sequence LBP
 Domain III VEVDMSGDLGWLLNLFHNQIESKFQKV (SEQ ID NO 22).

5. Several plasmids encoding LBP hybrid proteins were
 constructed for *in vitro* transcription/translation studies in which sequences
 selected from Domains II and/or III of BPI were inserted to replace the
 corresponding region of LBP₂₅. Conversely, other plasmids were
 constructed in which sequences selected from Domains II and/or III of LBP
 10 were inserted to replace sequences from the corresponding region of
 BPI₂₃.

First, plasmid pML131 was constructed comprising silent
 mutations in Domain II of BPI₂₃. Specifically, the nucleotide sequence
 between *Afl*III and *Bst*BI restriction sites in the BPI₂₃ coding region of
 15 pIC127 was replaced with the annealed oligonucleotides BPI-66: 5'-
 TTAAATTTTCGATATCCAACGCCAATATTAAGATCTCCGGAAAATG
 GAAGGCACAAAAGCGCTTCCTTAAGATGAG-3' (SEQ ID NO:23) and
 BPI-67: 5'-
 CTCATCTTAAGGAAGCGCTTTTGTGCCTTCCATTTTCCGGAGATCT
 20 TAATATTGGCGTTGGATATCGAAAAT (SEQ ID NO:24). This
 replacement changed the nucleotide sequence but not the amino acid
 sequence in the region between residues 77 and 100, and introduced
 additional restriction sites which could be used for site-directed mutagenesis
 (*Eco*RV, *Ssp*I, *Bgl*III, *Bsp*EI, *Eco*47III, and relocated *Afl*III).

25 In addition plasmid pML140 was constructed comprising
 silent mutations in Domain III of BPI₂₃. Specifically, the nucleotide
 sequence between *Pml*I and *Bst*BI restriction sites in the BPI₂₃ coding

region of pIC127 was replaced with the annealed oligonucleotides BPI-78:

5'-

GTGCACATTTCTGAAGAGCAAAGTGGGGTGGCTGATCCAATTGTTC
CACAAAAAATTGAGAGCGCGCTG-3' (SEQ ID NO:25) and BPI-79:

5'-

CGCAGCGCGCTCTCAATTTTTTTGTGGAACAATTGGATCAGCCACC
CCACTTTGCTCTTCGAAATGTGCAC-3' (SEQ ID NO:26). This

replacement changed the nucleotide sequence but not the amino acid
sequence in the region between residues 146 and 163, and introduced
additional restriction sites which could be used for site-directed mutagenesis
(relocated *Bst*BI, *Mun*I, and *Bss*HII):

A plasmid for *in vitro* transcription/translation studies
encoding the LBP hybrid protein [LBP(1-87)/BPI (88-100)/LBP(101-197)]
was constructed in which a portion of Domain II of BPI replaced the
corresponding LBP sequence. The hybrid protein comprised the first 87
amino acid residues of LBP, amino acid residues 88-100 of BPI and amino
acid residues 101-197 of LBP. The nucleotide sequence between *Ava*II and
*Ban*II restriction sites in the LBP₂₅ coding region of pML116 was replaced
with the annealed oligonucleotides BPI-74: 5'-

GTCAGCGGGAAATGGAAGGCACAAAAGAGATTTTAAAAATGCAG
GGCT-3' (SEQ ID NO:27) and BPI-75: 5'-

CTGCATTTTAAAAATCTCTTTTGTGCCTTCCATTTCCCGCT-3'

(SEQ ID NO:28). This replacement essentially changed the amino acid
sequence of residues 88-100 of LBP₂₅ from QGRWKVRKSFFKL (SEQ ID
NO:29) to SGKWKAQKRFLKM (SEQ ID NO:30). The resulting plasmid
was designated pML135.

A plasmid for *in vitro* transcription/translation studies encoding the LBP hybrid protein [LBP(1-146)/BPI(148-161)/LBP(160-197)] was constructed in which a portion of Domain III of BPI replaced the corresponding LBP sequence. The hybrid protein comprised the first 146 amino acid residues of LBP; amino acid residues 148-161 of BPI and amino acid residues 160-197 of LBP. Specifically, the nucleotide sequence between *AFIII* and *ScaI* restriction sites in the LBP₂₅ coding region of pML116 was replaced with the annealed oligonucleotides BPI-76: 5'-CATGTCGAAGAGCAAAGTGGGGTGGCTGATCCAACTCTTCCACAA
 5
 AAAAATTGAGTCCAAATTTTCAGAAAGT-3'(SEQ ID NO:31) and BPI-77: 5'-ACTTTCTGAAATTTGGACTCAATTTTTTTGTGGAAGAGTTGGATCA
 10
 GCCACCCCACTTTGCTCTTCGA-3'(SEQ ID NO:32). This replacement essentially changed the amino acid sequence of residues 147-159 of LBP₂₅ from GDLGWLLNLFHNQ (SEQ ID NO:33) to the corresponding BPI sequence KSKVGWLIQLFHKK (SEQ ID NO:34). This plasmid was designated pML137.

A plasmid for *in vitro* transcription/translation studies encoding the LBP hybrid protein [LBP(1-87)/BPI(88-100)/LBP(101-146)/BPI(148-161)/LBP(160-197)] was constructed in which portions of Domains II and III of BPI replaced the corresponding LBP sequences. The hybrid protein comprised the first 87 amino acid residues of LBP, amino acid residues 88-100 of BPI, amino acid residues 101-146 of LBP, amino acid residues 148-161 of BPI and amino acid residues 160-197 of LBP.
 20
 25
 Plasmid pML138 was constructed from pML135 and pML137 by replacing an ~292 bp *BanII-XhoI* fragment of the coding region of pML135 with the

corresponding fragment from pML137 to introduce the domain III mutations into the plasmid already containing the domain II mutations.

A plasmid for *in vitro* transcription/translation studies encoding the LBP hybrid protein [BPI(1-85)/LBP(86-99)/BPI(100-199)] was constructed in which a portion of Domain II of LBP replaced the corresponding BPI sequence. The hybrid protein comprised the first 85 amino acid residues of BPI, amino acid residues 86-99 of LBP and amino acid residues 100-199 of BPI. Specifically, the nucleotide sequence between *SspI* and *AflIII* restriction sites in the BPI₂₃ coding region of pML131 was replaced with the annealed oligonucleotides BPI-80: 5'-ATTCGTGTACAGGGCAGGTGGAAGGTGCGCAAGTCATTCT-3' (SEQ ID NO:35) and BPI-81: 5'-TTAAAGAATGACTTGCGCACCTTCCACCTGCCCTGTACACGAAT-3' (SEQ ID NO:36). This replacement essentially changed the amino acid sequence of residues 86-99 of BPI₂₃ from KISGKWKAQKRFLK (SEQ ID NO:37) to the corresponding LBP sequence RVQGRWKVRKSFFK. This plasmid was designated pML141 (SEQ ID NO:38).

A plasmid for *in vitro* transcription/translation studies encoding the LBP hybrid protein [BPI(1-147)/LBP(147-159)/BPI(162-199)] was constructed in which a portion of Domain III of LBP replaced the corresponding BPI sequence. The hybrid protein comprised the first 147 amino acid residues of BPI, amino acid residues 147-159 of LBP and amino acid residues 162-199 of BPI. The nucleotide sequence between *BstBI* and *BssHII* restriction sites in the BPI₂₃ coding region of pML140 was replaced with the annealed oligonucleotides BPI-82: 5'-CGGGAGACTTGGGGTGGCTGTTGAACCTCTTCCACAACCAGATTGAGAG-3' (SEQ ID NO:39) and BPI-83: 5'-

CGCGCTCTCAATCTGGTTGTGGAAGAGGTTCAACAGCCACCCCAA
GTCTCC-3' (SEQ ID NO:40). This replacement essentially changed the
amino acid sequence of residues 148-161 of BPI₂₃ from
KSKVGWLIQLFHKK (SEQ ID NO:41) to the corresponding LBP
-5 sequence GDLGWLLNLFHNQ (SEQ ID NO:42). This plasmid was
designated pML142.

A plasmid for *in vitro* transcription/translation studies
encoding the LBP hybrid protein [BPI(1-85)/LBP(86-99)/BPI(100-
147)/LBP(147-159)/BPI(162-199)] was constructed in which portions of
10 Domains II and III of LBP replaced the corresponding BPI sequences. The
hybrid protein comprised the first 85 amino acid residues of BPI, amino
acid residues 86-99 of LBP, amino acid residues 100-147 of BPI, amino
acid residues 147-159 of LBP and amino acid residues 162-199 of BPI.
pML143 can be constructed from pML141 and pML142 by replacing an
15 ~ 175 bp *PmII-XhoI* fragment in the coding region of pML141 with the
corresponding fragment from pML142 to introduce the domain III
mutations into the plasmid already containing the domain II mutations.
This plasmid was designated pML143.

The resulting plasmids are used as templates in the *in vitro*
20 transcription/translation reaction to generate LBP hybrid proteins.
Mammalian vectors for expression of the LBP hybrid proteins are produced
and the derivatives are expressed according to the methods disclosed
previously. The biological activities of these LBP hybrid proteins are then
tested according to the methods set out above.

EXAMPLE 23

LBP derivatives in the form of synthetic LBP peptides were prepared according to the methods of Merrifield, *J. Am. Chem. Soc.* 85: 2149 (1963) and Merrifield, *Anal Chem.* 38: 1905-1914 (1966) using an Applied Biosystems, Inc. Model 432 synthesizer. The resulting derivatives comprised portions of the LBP sequence corresponding to either of BPI Domain II or III. The LBP derivative designated LBP-1 consisted of residues 73 through 99 of LBP having the sequence GQGLSLSISDSSIRVQGRWKVRKSFFK (SEQ ID NO:43). The LBP derivative designated LBP-2 consisted of residues 140 through 161 of LBP and had the sequence DVEVDMSGDSGWLLNLFHNQIE (SEQ ID NO:44). The LBP derivatives were subjected to an *Limulus* Amoebocyte Lysate (LAL) assay using a quantitative chromogenic LAL kit (Whitaker Bioproducts, Inc., Walkersville, MD) to determine neutralization of LPS. LBP-1 was found to neutralize endotoxin in the LAL assay while LBP-2 did not.

Numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the foregoing description of the presently preferred embodiments thereof. Consequently, the only limitations which should be placed upon the scope of the present invention are those which appear in the appended claims.

60

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: XOMA CORPORATION
(B) STREET: 2910 Seventh Street
(C) CITY: Berkeley
(D) STATE: California
(E) COUNTRY: United States of America
(F) POSTAL CODE: 94710

(ii) TITLE OF INVENTION: Lipopolysaccharide Binding Protein
Derivatives

(iii) NUMBER OF SEQUENCES: 44

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
(B) STREET: 6300 Sears Tower, 233 South Wacker Drive
(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606-6402

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Smith, Jeffrey S.
(B) REGISTRATION NUMBER: 31,879
(C) REFERENCE/DOCKET NUMBER: 27129/32137

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 312/474-6300
(B) TELEFAX: 312/474-0448
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 591 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

SUBSTITUTE SHEET (RULE 26)

61

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..591

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "rLBP₂₅"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCC AAC CCC GGC TTG GTC GCC AGG ATC ACC GAC AAG GGA CTG CAG TAT	48
Ala Asn Pro Gly Leu Val Ala Arg Ile Thr Asp Lys Gly Leu Gln Tyr	
1 5 10 15	
GCG GCC CAG GAG GGG CTA TTG GCT CTG CAG AGT GAG CTG CTC AGG ATC	96
Ala Ala Gln Gly Leu Leu Ala Leu Gln Ser Glu Leu Leu Arg Ile	
20 25 30	
ACG CTG CCT GAC TTC ACC GGG GAC TTG AGG ATC CCC CAC GTC GGC CGT	144
Thr Leu Pro Asp Phe Thr Gly Asp Leu Arg Ile Pro His Val Gly Arg	
35 40 45	
GGG CGC TAT GAG TTC CAC AGC CTG AAC ATC CAC AGC TGT GAG CTG CTT	192
Gly Arg Tyr Glu Phe His Ser Leu Asn Ile His Ser Cys Glu Leu Leu	
50 55 60	
CAC TCT GCG CTG AGG CCT GTC CCT GGC CAG GGC CTG AGT CTC AGC ATC	240
His Ser Ala Leu Arg Pro Val Pro Gly Gln Gly Leu Ser Leu Ser Ile	
65 70 75 80	
TCC GAC TCC TCC ATC CGG GTC CAG GGC AGG TGG AAG GTG CGC AAG TCA	288
Ser Asp Ser Ser Ile Arg Val Gln Gly Arg Trp Lys Val Arg Lys Ser	
85 90 95	
TTC TTC AAA CTA CAG GGC TCC TTT GAT GTC AGT GTC AAG GGC ATC AGC	336
Phe Phe Lys Leu Gln Gly Ser Phe Asp Val Ser Val Lys Gly Ile Ser	
100 105 110	
ATT TCG GTC AAC CTC CTG TTG GGC AGC GAG TCC TCC GGG AGG CCC ACA	384
Ile Ser Val Asn Leu Leu Leu Gly Ser Glu Ser Ser Gly Arg Pro Thr	
115 120 125	
GTT ACT GCC TCC AGC TGC AGC AGT GAC ATC GCT GAC GTG GAG GTG GAC	432
Val Thr Ala Ser Ser Cys Ser Ser Asp Ile Ala Asp Val Glu Val Asp	
130 135 140	
ATG TCG GGA GAC TTG GGG TGG CTG TTG AAC CTC TTC CAC AAC CAG ATT	480
Met Ser Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe His Asn Gln Ile	
145 150 155 160	
GAG TCC AAG TTC CAG AAA GTA CTG GAG AGC AGG ATT TGC GAA ATG ATC	528
Glu Ser Lys Phe Gln Lys Val Leu Glu Ser Arg Ile Cys Glu Met Ile	

62

	165		170		175	
CAG AAA TCG GTG TCC TCC GAT CTA CAG CCT TAT CTC CAA ACT CTG CCA						576
Gln Lys Ser Val Ser Ser Asp Leu Gln Pro Tyr Leu Gln Thr Leu Pro						
	180		185		190	
GTT ACA ACA GAG ATT						591
Val Thr Thr Glu Ile						
	195					

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 197 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "rLBP₂₅"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala Asn Pro Gly Leu Val Ala Arg Ile Thr Asp Lys Gly Leu Gln Tyr	
1 5 10 15	
Ala Ala Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu Leu Arg Ile	
20 25 30	
Thr Leu Pro Asp Phe Thr Gly Asp Leu Arg Ile Pro His Val Gly Arg	
35 40 45	
Gly Arg Tyr Glu Phe His Ser Leu Asn Ile His Ser Cys Glu Leu Leu	
50 55 60	
His Ser Ala Leu Arg Pro Val Pro Gly Gln Gly Leu Ser Leu Ser Ile	
65 70 75 80	
Ser Asp Ser Ser Ile Arg Val Gln Gly Arg Trp Lys Val Arg Lys Ser	
85 90 95	
Phe Phe Lys Leu Gln Gly Ser Phe Asp Val Ser Val Lys Gly Ile Ser	
100 105 110	
Ile Ser Val Asn Leu Leu Leu Gly Ser Glu Ser Ser Gly Arg Pro Thr	
115 120 125	
Val Thr Ala Ser Ser Cys Ser Ser Asp Ile Ala Asp Val Glu Val Asp	
130 135 140	
Met Ser Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe His Asn Gln Ile	
145 150 155 160	

63

(2) INFORMATION FOR SEO ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1443 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..1443

(ix) FEATURE:

```
(A) NAME/KEY: mat_peptide
(B) LOCATION: 76..1443
```

(ix) FEATURE:

(A) NAME/KEY: misc feature

(D) OTHER INFORMATION: "rLBP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG	GGG	GCC	TTG	GCC	AGA	GCC	CTG	CCG	TCC	ATA	CTG	CTG	GCA	TTG	CTG	48
Met	Gly	Ala	Leu	Ala	Arg	Ala	Leu	Pro	Ser	Ile	Leu	Leu	Ala	Leu	Leu	
-25					-20					-15					-10	
CTT	ACG	TCC	ACC	CCA	GAG	GCT	CTG	GGT	GCC	AAC	CCC	GGC	TTG	GTC	GCC	96
Leu	Thr	Ser	Thr	Pro	Glu	Ala	Leu	Gly	Ala	Asn	Pro	Gly	Leu	Val	Ala	
				-5					1				5			
AGG	ATC	ACC	GAC	AAG	GGA	CTG	CAG	TAT	GCG	GCC	CAG	GAG	GGG	CTA	TTG	144
Arg	Ile	Thr	Asp	Lys	Gly	Leu	Gln	Tyr	Ala	Ala	Gln	Glu	Gly	Leu	Leu	
		10					15					20				
GCT	CTG	CAG	AGT	GAG	CTG	CTC	AGG	ATC	ACG	CTG	CCT	GAC	TTC	ACC	GGG	192
Ala	Leu	Gln	Ser	Glu	Leu	Leu	Arg	Ile	Thr	Leu	Pro	Asp	Phe	Thr	Gly	
	25					30					35					
GAC	TTG	AGG	ATC	CCC	CAC	GTC	GGC	CGT	GGG	CGC	TAT	GAG	TTC	CAC	AGC	240
Asp	Leu	Arg	Ile	Pro	His	Val	Gly	Arg	Gly	Arg	Tyr	Glu	Phe	His	Ser	
40					45					50					55	

64

CTG AAC ATC CAC AGC TGT GAG CTG CTT CAC TCT GCG CTG AGG CCT GTC	288
Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val	
60 65 70	
CCT GGC CAG GGC CTG AGT CTC AGC ATC TCC GAC TCC TCC ATC CGG GTC	336
Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val	
75 80 85	
CAG GGC AGG TGG AAG GTG CGC AAG TCA TTC TTC AAA CTA CAG GGC TCC	384
Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser	
90 95 100	
TTT GAT GTC AGT GTC AAG GGC ATC AGC ATT TCG GTC AAC CTC CTG TTG	432
Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu	
105 110 115	
GGC AGC GAG TCC TCC GGG AGG CCC ACA GTT ACT GCC TCC AGC TGC AGC	480
Gly Ser Glu Ser Ser Gly Arg Pro Thr Val Thr Ala Ser Ser Cys Ser	
120 125 130 135	
AGT GAC ATC GCT GAC GTG GAG GTG GAC ATG TCG GGA GAC TTG GGG TGG	528
Ser Asp Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Leu Gly Trp	
140 145 150	
CTG TTG AAC CTC TTC CAC AAC CAG ATT GAG TCC AAG TTC CAG AAA GTA	576
Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val	
155 160 165	
CTG GAG AGC AGG ATT TGC GAA ATG ATC CAG AAA TCG GTG TCC TCC GAT	624
Leu Glu Ser Arg Ile Cys Glu Met Ile Gln Lys Ser Val Ser Ser Asp	
170 175 180	
CTA CAG CCT TAT CTC CAA ACT CTG CCA GTT ACA ACA GAG ATT GAC AGT	672
Leu Gln Pro Tyr Leu Gln Thr Leu Pro Val Thr Thr Glu Ile Asp Ser	
185 190 195	
TTC GCC GAC ATT GAT TAT AGC TTA GTG GAA GCC CCT CGG GCA ACA GCC	720
Phe Ala Asp Ile Asp Tyr Ser Leu Val Glu Ala Pro Arg Ala Thr Ala	
200 205 210 215	
CAG ATG CTG GAG GTG ATG TTT AAG GGT GAA ATC TTT CAT CGT AAC CAC	768
Gln Met Leu Glu Val Met Phe Lys Gly Glu Ile Phe His Arg Asn His	
220 225 230	
CGT TCT CCA GTT ACC CTC CTT GCT GCA GTC ATG AGC CTT CCT GAG GAA	816
Arg Ser Pro Val Thr Leu Leu Ala Ala Val Met Ser Leu Pro Glu Glu	
235 240 245	
CAC AAC AAA ATG GTC TAC TTT GCC ATC TCG GAT TAT GTC TTC AAC ACG	864
His Asn Lys Met Val Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr	
250 255 260	
GCC AGC CTG GTT TAT CAT GAG GAA GGA TAT CTG AAC TTC TCC ATC ACA	912
Ala Ser Leu Val Tyr His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr	
265 270 275	

65

GAT GAG ATG ATA CCG CCT GAC TCT AAT ATC CGA CTG ACC ACC AAG TCC Asp Glu Met Ile Pro Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser 280 285 290 295	960
TTC CGA CCC TTC GTC CCA CGG TTA GCC AGG CTC TAC CCC AAC ATG AAC Phe Arg Pro Phe Val Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn 300 305 310	1008
CTG GAA CTC CAG GGA TCA GTG CCC TCT GCT CCG CTC CTG AAC TTC AGC Leu Glu Leu Gln Gly Ser Val Pro Ser Ala Pro Leu Leu Asn Phe Ser 315 320 325	1056
CCT GGG AAT CTG TCT GTG GAC CCC TAT ATG GAG ATA GAT GCC TTT GTG Pro Gly Asn Leu Ser Val Asp Pro Tyr Met Glu Ile Asp Ala Phe Val 330 335 340	1104
CTC CTG CCC AGC TCC AGC AAG GAG CCT GTC TTC CGG CTC AGT GTG GCC Leu Leu Pro Ser Ser Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala 345 350 355	1152
ACT AAT GTG TCC GCC ACC TTG ACC TTC AAT ACC AGC AAG ATC ACT GGG Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly 360 365 370 375	1200
TTC CTG AAG CCA GGA AAG GTA AAA GTG GAA CTG AAA GAA TCC AAA GTT Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val 380 385 390	1248
GGA CTA TTC AAT GCA GAG CTG TTG GAA GCG CTC CTC AAC TAT TAC ATC Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile 395 400 405	1296
CTT AAC ACC TTC TAC CCC AAG TTC AAT GAT AAG TTG GCC GAA GGC TTC Leu Asn Thr Phe Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe 410 415 420	1344
CCC CTT CCT CTG CTG AAG CGT GTT CAG CTC TAC GAC CTT GGG CTG CAG Pro Leu Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln 425 430 435	1392
ATC CAT AAG GAC TTC CTG TTC TTG GGT GCC AAT GTC CAA TAC ATG AGA Ile His Lys Asp Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg 440 445 450 455	1440
GTT Val	1443

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 481 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

66

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "rLBP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Gly Ala Leu Ala Arg Ala Leu Pro Ser Ile Leu Leu Ala Leu Leu
-25          -20          -15          -10

Leu Thr Ser Thr Pro Glu Ala Leu Gly Ala Asn Pro Gly Leu Val Ala
          -5              1              5

Arg Ile Thr Asp Lys Gly Leu Gln Tyr Ala Ala Gln Glu Gly Leu Leu
          10              15              20

Ala Leu Gln Ser Glu Leu Leu Arg Ile Thr Leu Pro Asp Phe Thr Gly
          25              30              35

Asp Leu Arg Ile Pro His Val Gly Arg Gly Arg Tyr Glu Phe His Ser
40          45          50          55

Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val
          60              65              70

Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val
          75              80              85

Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser
          90              95              100

Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu
105          110          115

Gly Ser Glu Ser Ser Gly Arg Pro Thr Val Thr Ala Ser Ser Cys Ser
120          125          130          135

Ser Asp Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Leu Gly Trp
          140          145          150

Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val
          155          160          165

Leu Glu Ser Arg Ile Cys Glu Met Ile Gln Lys Ser Val Ser Ser Asp
170          175          180

Leu Gln Pro Tyr Leu Gln Thr Leu Pro Val Thr Thr Glu Ile Asp Ser
185          190          195

Phe Ala Asp Ile Asp Tyr Ser Leu Val Glu Ala Pro Arg Ala Thr Ala
200          205          210          215

Gln Met Leu Glu Val Met Phe Lys Gly Glu Ile Phe His Arg Asn His

```

67

220										225					230				
Arg	Ser	Pro	Val	Thr	Leu	Leu	Ala	Ala	Val	Met	Ser	Leu	Pro	Glu	Glu				
			235						240					245					
His	Asn	Lys	Met	Val	Tyr	Phe	Ala	Ile	Ser	Asp	Tyr	Val	Phe	Asn	Thr				
		250					255					260							
Ala	Ser	Leu	Val	Tyr	His	Glu	Glu	Gly	Tyr	Leu	Asn	Phe	Ser	Ile	Thr				
	265					270					275								
Asp	Glu	Met	Ile	Pro	Pro	Asp	Ser	Asn	Ile	Arg	Leu	Thr	Thr	Lys	Ser				
280					285					290				295					
Phe	Arg	Pro	Phe	Val	Pro	Arg	Leu	Ala	Arg	Leu	Tyr	Pro	Asn	Met	Asn				
				300					305					310					
Leu	Glu	Leu	Gln	Gly	Ser	Val	Pro	Ser	Ala	Pro	Leu	Leu	Asn	Phe	Ser				
			315					320					325						
Pro	Gly	Asn	Leu	Ser	Val	Asp	Pro	Tyr	Met	Glu	Ile	Asp	Ala	Phe	Val				
		330					335					340							
Leu	Leu	Pro	Ser	Ser	Ser	Lys	Glu	Pro	Val	Phe	Arg	Leu	Ser	Val	Ala				
	345					350					355								
Thr	Asn	Val	Ser	Ala	Thr	Leu	Thr	Phe	Asn	Thr	Ser	Lys	Ile	Thr	Gly				
360					365					370				375					
Phe	Leu	Lys	Pro	Gly	Lys	Val	Lys	Val	Glu	Leu	Lys	Glu	Ser	Lys	Val				
				380					385					390					
Gly	Leu	Phe	Asn	Ala	Glu	Leu	Leu	Glu	Ala	Leu	Leu	Asn	Tyr	Tyr	Ile				
			395					400					405						
Leu	Asn	Thr	Phe	Tyr	Pro	Lys	Phe	Asn	Asp	Lys	Leu	Ala	Glu	Gly	Phe				
		410					415					420							
Pro	Leu	Pro	Leu	Leu	Lys	Arg	Val	Gln	Leu	Tyr	Asp	Leu	Gly	Leu	Gln				
	425					430					435								
Ile	His	Lys	Asp	Phe	Leu	Phe	Leu	Gly	Ala	Asn	Val	Gln	Tyr	Met	Arg				
440					445						450			455					
Val																			

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

68

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP-Bsm"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATGCAGCC AACCCCGGCT TGGTCGCCA

29

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP-2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTCGAGCTAA ATCTCTGTTG TAACTGGC

28

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP-3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CATGTCGACA CCATGGGGGC CTTG

24

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

69

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (D) OTHER INFORMATION: "LBP-4"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
CATGCCGCGG TCAAACCTCTC ATGTA
- (2) INFORMATION FOR SEQ ID NO:9:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: DNA
 - (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (D) OTHER INFORMATION: "LBP 241-245"
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
GTCATGAGCC TTCCT
 - (2) INFORMATION FOR SEQ ID NO:10:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
 - (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (D) OTHER INFORMATION: "LBP 241-245"
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
Val Met Ser Leu Pro
1 5
 - (2) INFORMATION FOR SEQ ID NO:11:

25

15

70

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1813 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 31..1491

- (ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 124..1491

- (ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (D) OTHER INFORMATION: "BPI"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CAGGCCTGA GGTTTGGCA GCTCTGGAGG ATG AGA GAG AAC ATG GCC AGG GGC	54
Met Arg Glu Asn Met Ala Arg Gly	
-31 -30 -25	
CCT TGC AAC GCG CCG AGA TGG GTG TCC CTG ATG GTG CTC GTC GCC ATA	102
Pro Cys Asn Ala Pro Arg Trp Val Ser Leu Met Val Leu Val Ala Ile	
-20 -15 -10	
GGC ACC GCC GTG ACA GCG GCC GTC AAC CCT GGC GTC GTG GTC AGG ATC	150
Gly Thr Ala Val Thr Ala Ala Val Asn Pro Gly Val Val Val Arg Ile	
-5 1 5	
TCC CAG AAG GGC CTG GAC TAC GCC AGC CAG CAG GGG ACG GCC GCT CTG	198
Ser Gln Lys Gly Leu Asp Tyr Ala Ser Gln Gln Gly Thr Ala Ala Leu	
10 15 20 25	
CAG AAG GAG CTG AAG AGG ATC AAG ATT CCT GAC TAC TCA GAC AGC TTT	246
Gln Lys Glu Leu Lys Arg Ile Lys Ile Pro Asp Tyr Ser Asp Ser Phe	
30 35 40	
AAG ATC AAG CAT CTT GGG AAG GGG CAT TAT AGC TTC TAC AGC ATG GAC	294
Lys Ile Lys His Leu Gly Lys Gly His Tyr Ser Phe Tyr Ser Met Asp	
45 50 55	
ATC CGT GAA TTC CAG CTT CCC AGT TCC CAG ATA AGC ATG GTG CCC AAT	342
Ile Arg Glu Phe Gln Leu Pro Ser Ser Gln Ile Ser Met Val Pro Asn	
60 65 70	
GTG GGC CTT AAG TTC TCC ATC AGC AAC GCC AAT ATC AAG ATC AGC GGC	390
Val Gly Leu Lys Phe Ser Ile Ser Asn Ala Asn Ile Lys Ile Ser Gly	
75 80 85	

71

AAA TGG AAG GCA CAA AAG AGA TTC TTA AAA ATG AGC GGC AAT TTT GAC	438
Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Met Ser Gly Asn Phe Asp	
90 95 100 105	
CTG AGC ATA GAA GGC ATG TCC ATT TCG GCT GAT CTG AAG CTG GGC AGT	486
Leu Ser Ile Glu Gly Met Ser Ile Ser Ala Asp Leu Lys Leu Gly Ser	
110 115 120	
AAC CCC ACG TCA GGC AAG CCC ACC ATC ACC TGC TCC AGC TGC AGC AGC	534
Asn Pro Thr Ser Gly Lys Pro Thr Ile Thr Cys Ser Ser Cys Ser Ser	
125 130 135	
CAC ATC AAC AGT GTC CAC GTG CAC ATC TCA AAG AGC AAA GTC GGG TGG	582
His Ile Asn Ser Val His Val His Ile Ser Lys Ser Lys Val Gly Trp	
140 145 150	
CTG ATC CAA CTC TTC CAC AAA AAA ATT GAG TCT GCG CTT CGA AAC AAG	630
Leu Ile Gln Leu Phe His Lys Lys Ile Glu Ser Ala Leu Arg Asn Lys	
155 160 165	
ATG AAC AGC CAG GTC TGC GAG AAA GTG ACC AAT TCT GTA TCC TCC AAG	678
Met Asn Ser Gln Val Cys Glu Lys Val Thr Asn Ser Val Ser Ser Lys	
170 175 180 185	
CTG CAA CCT TAT TTC CAG ACT CTG CCA GTA ATG ACC AAA ATA GAT TCT	726
Leu Gln Pro Tyr Phe Gln Thr Leu Pro Val Met Thr Lys Ile Asp Ser	
190 195 200	
GTG GCT GGA ATC AAC TAT GGT CTG GTG GCA CCT CCA GCA ACC ACG GCT	774
Val Ala Gly Ile Asn Tyr Gly Leu Val Ala Pro Pro Ala Thr Thr Ala	
205 210 215	
GAG ACC CTG GAT GTA CAG ATG AAG GGG GAG TTT TAC AGT GAG AAC CAC	822
Glu Thr Leu Asp Val Gln Met Lys Gly Glu Phe Tyr Ser Glu Asn His	
220 225 230	
CAC AAT CCA CCT CCC TTT GCT CCA CCA GTG ATG GAG TTT CCC GCT GCC	870
His Asn Pro Pro Pro Phe Ala Pro Pro Val Met Glu Phe Pro Ala Ala	
235 240 245	
CAT GAC CGC ATG GTA TAC CTG GGC CTC TCA GAC TAC TTC TTC AAC ACA	918
His Asp Arg Met Val Tyr Leu Gly Leu Ser Asp Tyr Phe Phe Asn Thr	
250 255 260 265	
GCC GGG CTT GTA TAC CAA GAG GCT GGG GTC TTG AAG ATG ACC CTT AGA	966
Ala Gly Leu Val Tyr Gln Glu Ala Gly Val Leu Lys Met Thr Leu Arg	
270 275 280	
GAT GAC ATG ATT CCA AAG GAG TCC AAA TTT CGA CTG ACA ACC AAG TTC	1014
Asp Asp Met Ile Pro Lys Glu Ser Lys Phe Arg Leu Thr Thr Lys Phe	
285 290 295	
TTT GGA ACC TTC CTA CCT GAG GTG GCC AAG AAG TTT CCC AAC ATG AAG	1062
Phe Gly Thr Phe Leu Pro Glu Val Ala Lys Lys Phe Pro Asn Met Lys	
300 305 310	

72

ATA CAG ATC CAT GTC TCA GCC TCC ACC CCG CCA CAC CTG TCT GTG CAG	1110
Ile Gln Ile His Val Ser Ala Ser Thr Pro Pro His Leu Ser Val Gln	
315 320 325	
CCC ACC GGC CTT ACC TTC TAC CCT GCC GTG GAT GTC CAG GCC TTT GCC	1158
Pro Thr Gly Leu Thr Phe Tyr Pro Ala Val Asp Val Gln Ala Phe Ala	
330 335 340 345	
GTC CTC CCC AAC TCC TCC CTG GCT TCC CTC TTC CTG ATT GGC ATG CAC	1206
Val Leu Pro Asn Ser Ser Leu Ala Ser Leu Phe Leu Ile Gly Met His	
350 355 360	
ACA ACT GGT TCC ATG GAG GTC AGC GCC GAG TCC AAC AGG CTT GTT GGA	1254
Thr Thr Gly Ser Met Glu Val Ser Ala Glu Ser Asn Arg Leu Val Gly	
365 370 375	
GAG CTC AAG CTG GAT AGG CTG CTC CTG GAA CTG AAG CAC TCA AAT ATT	1302
Glu Leu Lys Leu Asp Arg Leu Leu Leu Glu Leu Lys His Ser Asn Ile	
380 385 390	
GGC CCC TTC CCG GTT GAA TTG CTG CAG GAT ATC ATG AAC TAC ATT GTA	1350
Gly Pro Phe Pro Val Glu Leu Leu Gln Asp Ile Met Asn Tyr Ile Val	
395 400 405	
CCC ATT CTT GTG CTG CCC AGG GTT AAC GAG AAA CTA CAG AAA GGC TTC	1398
Pro Ile Leu Val Leu Pro Arg Val Asn Glu Lys Leu Gln Lys Gly Phe	
410 415 420 425	
CCT CTC CCG ACG CCG GCC AGA GTC CAG CTC TAC AAC GTA GTG CTT CAG	1446
Pro Leu Pro Thr Pro Ala Arg Val Gln Leu Tyr Asn Val Val Leu Gln	
430 435 440	
CCT CAC CAG AAC TTC CTG CTG TTC GGT GCA GAC GTT GTC TAT AAA	1491
Pro His Gln Asn Phe Leu Leu Phe Gly Ala Asp Val Val Tyr Lys	
445 450 455	
TGAAGGCACC AGGGGTGCCG GGGGCTGTCA GCCGCACCTG TTCCTGATGG GCTGTGGGGC	1551
ACCGGCTGCC TTTCCCAGG GAATCCTCTC CAGATCTTAA CCAAGAGCCC CTGCAAACT	1611
TCTTCGACTC AGATTCAGAA ATGATCTAAA CACGAGGAAA CATTATTCAT TGGAAAAGTG	1671
CATGGTGTGT ATTTTAGGGA TTATGAGCTT CTTTCAAGGG CTAAGGCTGC AGAGATATTT	1731
CCTCCAGGAA TCGTGTTTCA ATTGTAACCA AGAAATTTCC ATTTGTGCTT CATGAAAAAA	1791
AACTTCTGGT TTTTTCATG TG	1813

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 487 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

73

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Arg Glu Asn Met Ala Arg Gly Pro Cys Asn Ala Pro Arg Trp Val
-31 -30          -25          -20

Ser Leu Met Val Leu Val Ala Ile Gly Thr Ala Val Thr Ala Ala Val
-15          -10          -5          1

Asn Pro Gly Val Val Val Arg Ile Ser Gln Lys Gly Leu Asp Tyr Ala
          5          10          15

Ser Gln Gln Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile Lys
          20          25          30

Ile Pro Asp Tyr Ser Asp Ser Phe Lys Ile Lys His Leu Gly Lys Gly
          35          40          45

His Tyr Ser Phe Tyr Ser Met Asp Ile Arg Glu Phe Gln Leu Pro Ser
          50          55          60          65

Ser Gln Ile Ser Met Val Pro Asn Val Gly Leu Lys Phe Ser Ile Ser
          70          75          80

Asn Ala Asn Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe
          85          90          95

Leu Lys Met Ser Gly Asn Phe Asp Leu Ser Ile Glu Gly Met Ser Ile
          100          105          110

Ser Ala Asp Leu Lys Leu Gly Ser Asn Pro Thr Ser Gly Lys Pro Thr
          115          120          125

Ile Thr Cys Ser Ser Cys Ser Ser His Ile Asn Ser Val His Val His
          130          135          140          145

Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
          150          155          160

Ile Glu Ser Ala Leu Arg Asn Lys Met Asn Ser Gln Val Cys Glu Lys
          165          170          175

Val Thr Asn Ser Val Ser Ser Lys Leu Gln Pro Tyr Phe Gln Thr Leu
          180          185          190

Pro Val Met Thr Lys Ile Asp Ser Val Ala Gly Ile Asn Tyr Gly Leu
          195          200          205

Val Ala Pro Pro Ala Thr Thr Ala Glu Thr Leu Asp Val Gln Met Lys
          210          215          220          225

```

Gly	Glu	Phe	Tyr	Ser	Glu	Asn	His	His	Asn	Pro	Pro	Pro	Phe	Ala	Pro	230	235	240
Pro	Val	Met	Glu	Phe	Pro	Ala	Ala	His	Asp	Arg	Met	Val	Tyr	Leu	Gly	245	250	255
Leu	Ser	Asp	Tyr	Phe	Phe	Asn	Thr	Ala	Gly	Leu	Val	Tyr	Gln	Glu	Ala	260	265	270
Gly	Val	Leu	Lys	Met	Thr	Leu	Arg	Asp	Asp	Met	Ile	Pro	Lys	Glu	Ser	275	280	285
Lys	Phe	Arg	Leu	Thr	Thr	Lys	Phe	Phe	Gly	Thr	Phe	Leu	Pro	Glu	Val	290	295	300
Ala	Lys	Lys	Phe	Pro	Asn	Met	Lys	Ile	Gln	Ile	His	Val	Ser	Ala	Ser	310	315	320
Thr	Pro	Pro	His	Leu	Ser	Val	Gln	Pro	Thr	Gly	Leu	Thr	Phe	Tyr	Pro	325	330	335
Ala	Val	Asp	Val	Gln	Ala	Phe	Ala	Val	Leu	Pro	Asn	Ser	Ser	Leu	Ala	340	345	350
Ser	Leu	Phe	Leu	Ile	Gly	Met	His	Thr	Thr	Gly	Ser	Met	Glu	Val	Ser	355	360	365
Ala	Glu	Ser	Asn	Arg	Leu	Val	Gly	Glu	Leu	Lys	Leu	Asp	Arg	Leu	Leu	370	375	380
Leu	Glu	Leu	Lys	His	Ser	Asn	Ile	Gly	Pro	Phe	Pro	Val	Glu	Leu	Leu	390	395	400
Gln	Asp	Ile	Met	Asn	Tyr	Ile	Val	Pro	Ile	Leu	Val	Leu	Pro	Arg	Val	405	410	415
Asn	Glu	Lys	Leu	Gln	Lys	Gly	Phe	Pro	Leu	Pro	Thr	Pro	Ala	Arg	Val	420	425	430
Gln	Leu	Tyr	Asn	Val	Val	Leu	Gln	Pro	His	Gln	Asn	Phe	Leu	Leu	Phe	435	440	445
Gly	Ala	Asp	Val	Val	Tyr	Lys										450	455	

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

75

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI-18"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAGCATCTTG GGAAGGGG

18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI-11"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TATTTTGGTC ATTACTGGCA GAGT

24

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP15"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCCACAGTCA CGTGCTCCAG CTGCA

25

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

76

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP16"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCTGGAGCAC GTGACTGTGG GCC

23

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI Domain I"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Ser Gln Gln Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile
 1 5 10 15

Lys Pro Asp Tyr Ser Asp Ser Phe Lys Ile Lys His
 20 25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI Domain II"

77

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```

Ser Ser Gln Ile Ser Met Val Pro Asn Val Gly Leu Lys Phe Ser Ile
1           5           10           15

Ser Asn Ala Asn Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg
          20           25           30

Phe Leu Lys
          35

```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI Domain III"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

Val His Val His Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln Leu
1           5           10           15

Phe His Lys Lys Ile Glu Ser Ala Leu Arg Asn Lys
          20           25

```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP Domain I"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```

Ala Ala Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu Leu Arg Ile
1           5           10           15

```

78

Thr Leu Pro Asp Phe Thr Gly Asp Leu Arg Ile Pro His
 20 25

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP Domain II"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

His Ser Ala Leu Arg Pro Val Pro Gly Gln Gly Leu Ser Leu Ser Ile
 1 5 10 15
 Ser Asp Ser Ser Ile Arg Val Gln Gly Arg Trp Lys Val Arg Lys Ser
 20 25 30
 Phe Phe Lys
 35

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP Domain III"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Val Glu Val Asp Met Ser Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe
 1 5 10 15
 His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val
 20 25

(2) INFORMATION FOR SEQ ID NO:23:

79

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 76 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TTAAATTTTC GATATCCAAC GCCAATATTA AGATCTCCGG AAAATGGAAG GCACAAAAGC 60

GCTTCCTTAA GATGAG 76

- (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 72 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTCATCTTAA GGAAGCGCTT TTGTGCCTTC CATTTTCCGG AGATCTTAAT ATTGGCGTTG 60

GATATCGAAA AT 72

- (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 69 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GTGCACATTT CGAAGAGCAA AGTGGGGTGG CTGATCCAAT TGTTCCACAA AAAAATTGAG 60

AGCGCGCTG 69

- (2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 71 base pairs

80

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGCAGCGCGC TCTCAATTTT TTTGTGGAAC AATTGGATCA GCCACCCAC TTTGCTCTTC 60
GAAATGTGCA C 71

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 49 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTCAGCGGGA AATGGAAGGC ACAAAGAGA TTTTAAAAA TGCAGGGCT 49

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CTGCATTTTT AAAATCTCT TTTGTGCCTT CCATTCCCG CT 42

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

81

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu
 1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Met
 1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 72 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CATGTCGAAG AGCAAAGTGG GGTGGCTGAT CCAACTCTTC CACAAAAAAA TTGAGTCCAA 60
 ATTCAGAAA GT 72

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 68 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ACTTTCTGAA ATTTGGACTC AATTTTTTTG TGAAGAGTT GGATCAGCCA CCCCACTTTG 60

82

CTCTTCGA

68

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe His Asn Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATTCTGTAC AGGGCAGGTG GAAGGTGCGC AAGTCATTCT

40

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 44 base pairs

83

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TTAAGAATG ACTTGCGCAC CTTCCACCTG CCCTGTACAC GAAT

44

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Arg Val Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 49 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CGGGAGACTT GGGGTGGCTG TTGAACCTCT TCCACAACCA GATTGAGAG

49

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CGCGCTCTCA ATCTGGTTGT GGAAGAGGTT CAACAGCCAC CCCAAGTCTC C

51

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe His Asn Gln
1 5 10

85

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val Gln
1 5 10 15

Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys
20 25

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asp Val Glu Val Asp Met Ser Gly Asp Ser Gly Trp Leu Leu Asn Leu
1 5 10 15

Phe His Asn Gln Ile Glu
20

WHAT IS CLAIMED IS:

1. An LBP protein derivative having an ability to bind to LPS and lacking CD14-mediated immunostimulatory properties.

2. The derivative according to claim 1 which has a molecular weight of about 25 kD.

3. The derivative according to claim 1 consisting of a portion of the amino-terminal half of LBP.

4. The derivative according to claim 3 consisting of amino acids 1 through 197 of SEQ ID NO:1.

5. The derivative according to claim 1 which is LBP (1-197) (Cys131).

6. An LBP derivative hybrid protein having an ability to bind to LPS and lacking CD14-mediated immunostimulatory properties and characterized by the presence of at least a portion of an LPS binding domain of BPI selected from the group consisting of:

ASQQGTAALQKELKRIKPDYSDSFKIKH (SEQ ID NO:17);

SSQISMVPNVGLKFSISNANIKISGKWKAQKRFLK (SEQ ID NO:18);

and VHVHISKSKVGWLIQLFHKKIESALRNK (SEQ ID NO:19).

87

7. The hybrid protein according to claim 6 characterized by the presence of at least a portion of an LPS binding domain of LBP selected from the group consisting of:

AAQEGLLALQSELLRITLPDFTGDLRIPH (SEQ IS NO:20);

5 HSALRPVPGQGLSLSISDSSIRVQGRWKVRKSFFK (SEQ ID NO:21);

and

VEVDMSGDLGWLLNLFHNQIESKFQKV (SEQ ID NO 22).

8. The hybrid protein according to claim 6 consisting of
10 a portion of the amino-terminal half of LBP/and a portion of the amino-terminal half of BPI.

9. The hybrid protein according to claim 6 which is selected from the group consisting of LBP(1-43)/BPI(44-199), BPI(1-159)/LBP(158-197), LBP(1-43)/BPI(44-159)/LBP(158-197), BPI(1-137)/LBP(137-197), BPI(1-25)/LBP(26-135)/BPI(137-199), [LBP(1-87)/BPI(88-100)/LBP(101-197)], [LBP(1-146)/BPI(148-161)/LBP(160-197)], [LBP(1-87)/BPI(88-100)/LBP(101-146)/BPI(148-161)/LBP(160-197)], [BPI(1-85)/LBP(86-99)/BPI(100-199)], [BPI(1-147)/LBP(147-159)/BPI(162-199)], and [BPI(1-85)/LBP(86-99)/BPI(100-147)/LBP(147-159)/BPI(162-199)].

10. The LBP derivative hybrid protein having an ability to bind LPS and lacking CD-14 mediated immunostimulatory properties
25 which is an LBP/IgG fusion protein.

11. A pharmaceutical composition comprising an LBP protein derivative or LBP derivative hybrid protein according claim 1, 6 or 10 and a pharmaceutically acceptable diluent, adjuvant or carrier.

5 12. A DNA sequence encoding an LBP derivative or LBP derivative hybrid or protein according to claim 1, 6 or 10.

13. A DNA vector comprising the DNA sequence according to claim 12.

10 14. A host cell stably transformed or transfected with a DNA sequence according to claim 12 in a manner allowing expression in the host cell of the protein encoded thereby.

15 15. A method of treating a gram-negative bacterial infection and the sequelae thereof comprising administering an LBP protein derivative or LBP derivative hybrid protein according to claim 1, 6 or 10.

20 16. The method of claim 15 wherein the LBP protein derivative or LBP derivative hybrid protein is administered at a dosage of from about 0.1 mg/kg to about 100 mg/kg of body weight.

25 17. The use of an LBP protein derivative or an LBP derivative hybrid protein claim 1, 6 or 10 in the manufacture of a medicament for the treatment of gram negative bacterial infection and the sequelae thereof.

1/21

LBP25 Sequence

1 GCC AAC CCC GGC TTG GTC GCC AGG ATC ACC GAC AAG GGA CTG CAG TAT GCG GCC
 1▶Ala Asn Pro Gly Leu Val Ala Arg Ile Thr Asp Lys Gly Leu Gln Tyr Ala Ala

 55 CAG GAG GGG CTA TTG GCT CTG CAG AGT GAG CTG CTC AGG ATC ACG CTG CCT GAC
 19▶Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu Leu Arg Ile Thr Leu Pro Asp

 109 TTC ACC GGG GAC TTG AGG ATC CCC CAC GTC GGC CGT GGG CGC TAT GAG TTC CAC
 37▶Phe Thr Gly Asp Leu Arg Ile Pro His Val Gly Arg Gly Arg Tyr Glu Phe His

 163 AGC CTG AAC ATC CAC AGC TGT GAG CTG CTT CAC TCT GCG CTG AGG CCT GTC CCT
 55▶Ser Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val Pro

 217 GGC CAG GGC CTG AGT CTC AGC ATC TCC GAC TCC TCC ATC CGG GTC CAG GGC AGG
 73▶Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val Gln Gly Arg

 271 TGG AAG GTG CGC AAG TCA TTC TTC AAA CTA CAG GGC TCC TTT GAT GTC AGT GTC
 91▶Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser Phe Asp Val Ser Val

 325 AAG GGC ATC AGC ATT TCG GTC AAC CTC CTG TTG GGC AGC GAG TCC TCC GGC AGG
 109▶Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu Gly Ser Glu Ser Ser Gly Arg

 379 CCC ACA GTT ACT GCC TCC AGC TGC AGC AGT GAC ATC GCT GAC GTG GAG GTG GAC
 127▶Pro Thr Val Thr Ala Ser Ser Cys Ser Ser Asp Ile Ala Asp Val Glu Val Asp

 433 ATG TCG GGA GAC TTG GGG TGG CTG TTG AAC CTC TTC CAC AAC CAG ATT GAG TCC
 145▶Met Ser Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser

 487 AAG TTC CAG AAA GTA CTG GAG AGC AGG ATT TGC GAA ATG ATC CAG AAA TCG GTG
 163▶Lys Phe Gln Lys Val Leu Glu Ser Arg Ile Cys Glu Met Ile Gln Lys Ser Val

 541 TCC TCC GAT CTA CAG CCT TAT CTC CAA ACT CTG CCA GTT ACA ACA GAG ATT
 181▶Ser Ser Asp Leu Gln Pro Tyr Leu Gln Thr Leu Pro Val Thr Thr Glu Ile

FIGURE 1

2 / 21

LBP Sequence

1 ATG GGG GCC TTG GCC AGA GCC CTG CCG TCC ATA CTG CTG GCA TTG CTG CTT ACG
 55 TCC ACC CCA GAG GCT CTG GGT GCC AAC CCC GGC TTG GTC GCC AGG ATC ACC GAC
 109 AAG GGA CTG CAG TAT GCG GCC CAG GAG GGG CTA TTG GCT CTG CAG AGT GAG CTG
 120 Lys Gly Leu Gln Tyr Ala Ala Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu
 163 CTC AGG ATC ACG CTG CCT GAC TTC ACC GGG GAC TTG AGG ATC CCC CAC GTC GGC
 300 Leu Arg Ile Thr Leu Pro Asp Phe Thr Gly Asp Leu Arg Ile Pro His Val Gly
 217 CGT GGG CGC TAT GAG TTC CAC AGC CTG AAC ATC CAC AGC TGT GAG CTG CTT CAC
 480 Arg Gly Arg Tyr Glu Phe His Ser Leu Asn Ile His Ser Cys Glu Leu Leu His
 271 TCT GCG CTG AGG CCT GTC CCT GGC CAG GGC CTG AGT CTC AGC ATC TCC GAC TCC
 660 Ser Ala Leu Arg Pro Val Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser
 325 TCC ATC CGG GTC CAG GGC AGG TGG AAG GTG CGC AAG TCA TTC TTC AAA CTA CAG
 840 Ser Ile Arg Val Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln
 379 GGC TCC TTT GAT GTC AGT GTC AAG GGC ATC AGC ATT TCG GTC AAC CTC CTG TTG
 1020 Gly Ser Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu
 433 GGC AGC GAG TCC TCC GGG AGG CCC ACA GTT ACT GCC TCC AGC TGC AGC AGT GAC
 1200 Gly Ser Glu Ser Ser Gly Arg Pro Thr Val Thr Ala Ser Ser Cys Ser Ser Asp
 487 ATC GCT GAC GTG GAG GTG GAC ATG TCG GGA GAC TTG GGG TGG CTG TTG AAC CTC
 1380 Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Leu Gly Trp Leu Leu Asn Leu
 541 TTC CAC AAC CAG ATT GAG TCC AAG TTC CAG AAA GTA CTG GAG AGC AGG ATT TGC
 1560 Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val Leu Glu Ser Arg Ile Cys
 595 GAA ATG ATC CAG AAA TCG GTG TCC TCC GAT CTA CAG CCT TAT CTC CAA ACT CTG
 1740 Glu Met Ile Gln Lys Ser Val Ser Ser Asp Leu Gln Pro Tyr Leu Gln Thr Leu
 649 CCA GTT ACA ACA GAG ATT GAC AGT TTC GCC GAC ATT GAT TAT AGC TTA GTG GAA
 1920 Pro Val Thr Thr Glu Ile Asp Ser Phe Ala Asp Ile Asp Tyr Ser Leu Val Glu
 703 GCC CCT CGG GCA ACA GCC CAG ATG CTG GAG GTG ATG TTT AAG GGT GAA ATC TTT
 2100 Ala Pro Arg Ala Thr Ala Gln Met Leu Glu Val Met Phe Lys Gly Glu Ile Phe
 757 CAT CGT AAC CAC CGT TCT CCA GTT ACC CTC CTT GCT GCA GTC ATG AGC CTT CCT
 2280 His Arg Asn His Arg Ser Pro Val Thr Leu Leu Ala Ala Val Met Ser Leu Pro
 811 GAG GAA CAC AAC AAA ATG GTC TAC TTT GCC ATC TCG GAT TAT GTC TTC AAC ACG
 2460 Glu Glu His Asn Lys Met Val Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr

FIGURE 2A

SUBSTITUTE SHEET (RULE 26)

3 / 21

365 GCC AGC CTG GTT TAT CAT GAG GAA GGA TAT CTG AAC TTC TCC ATC ACA GAT GAC
264▶Ala Ser Leu Val Tyr His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr Asp Asp

919 ATG ATA CCG CCT GAC TCT AAT ATC CGA CTG ACC ACC AAG TCC TTC CGA CCC TTC
282▶Met Ile Pro Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser Phe Arg Pro Phe

973 GTC CCA CGG TTA GCC AGG CTC TAC CCC AAC ATG AAC CTG GAA CTC CAG GGA TCA
300▶Val Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn Leu Glu Leu Gln Gly Ser

1027 GTG CCC TCT GCT CCG CTC CTG AAC TTC AGC CCT GGG AAT CTG TCT GTG GAC CCC
318▶Val Pro Ser Ala Pro Leu Leu Asn Phe Ser Pro Gly Asn Leu Ser Val Asp Pro

1081 TAT ATG GAG ATA GAT GCC TTT GTG CTC CTG CCC AGC TCC AGC AAG GAG CCT GTC
336▶Tyr Met Glu Ile Asp Ala Phe Val Leu Leu Pro Ser Ser Ser Lys Glu Pro Val

1135 TTC CGG CTC AGT GTG GCC ACT AAT GTG TCC GCC ACC TTG ACC TTC AAT ACC AGC
354▶Phe Arg Leu Ser Val Ala Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser

1189 AAG ATC ACT GGG TTC CTG AAG CCA GGA AAG GTA AAA GTG GAA CTG AAA GAA TCC
372▶Lys Ile Thr Gly Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser

1243 AAA GTT GGA CTA TTC AAT GCA GAG CTG TTG GAA GCG CTC CTC AAC TAT TAC ATC
390▶Lys Val Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile

1297 CTT AAC ACC TTC TAC CCC AAG TTC AAT GAT AAG TTG GCC GAA GGC TTC CCC CTT
408▶Leu Asn Thr Phe Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe Pro Leu

1351 CCT CTG CTG AAG CGT GTT CAG CTC TAC GAC CTT GGG CTG CAG ATC CAT AAG GAC
426▶Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln Ile His Lys Asp

1405 TTC CTG TTC TTG GGT GCC AAT GTC CAA TAC ATG AGA GTT
444▶Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg Val

FIGURE 2B

4 / 21

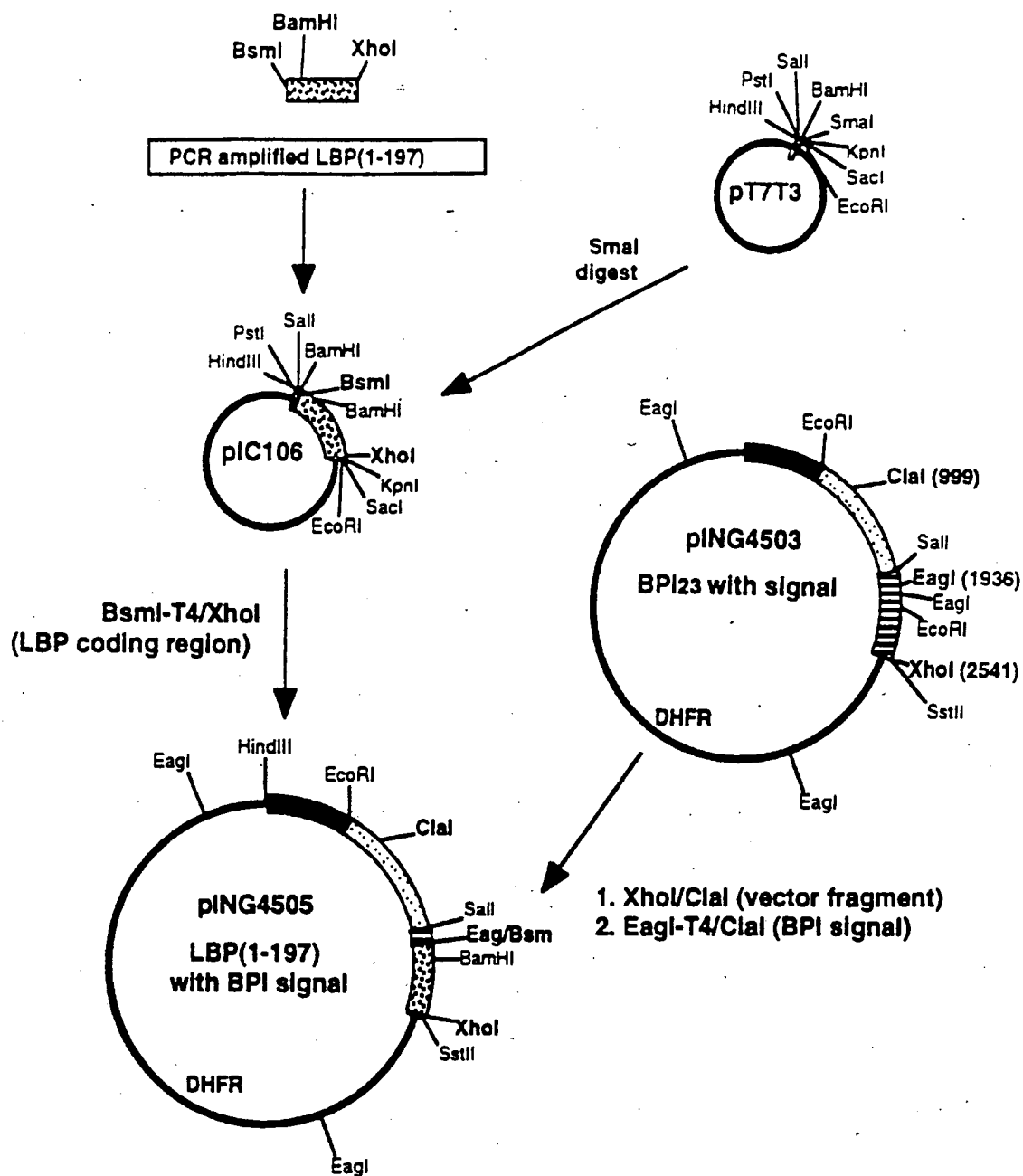


FIGURE 3

5 / 21

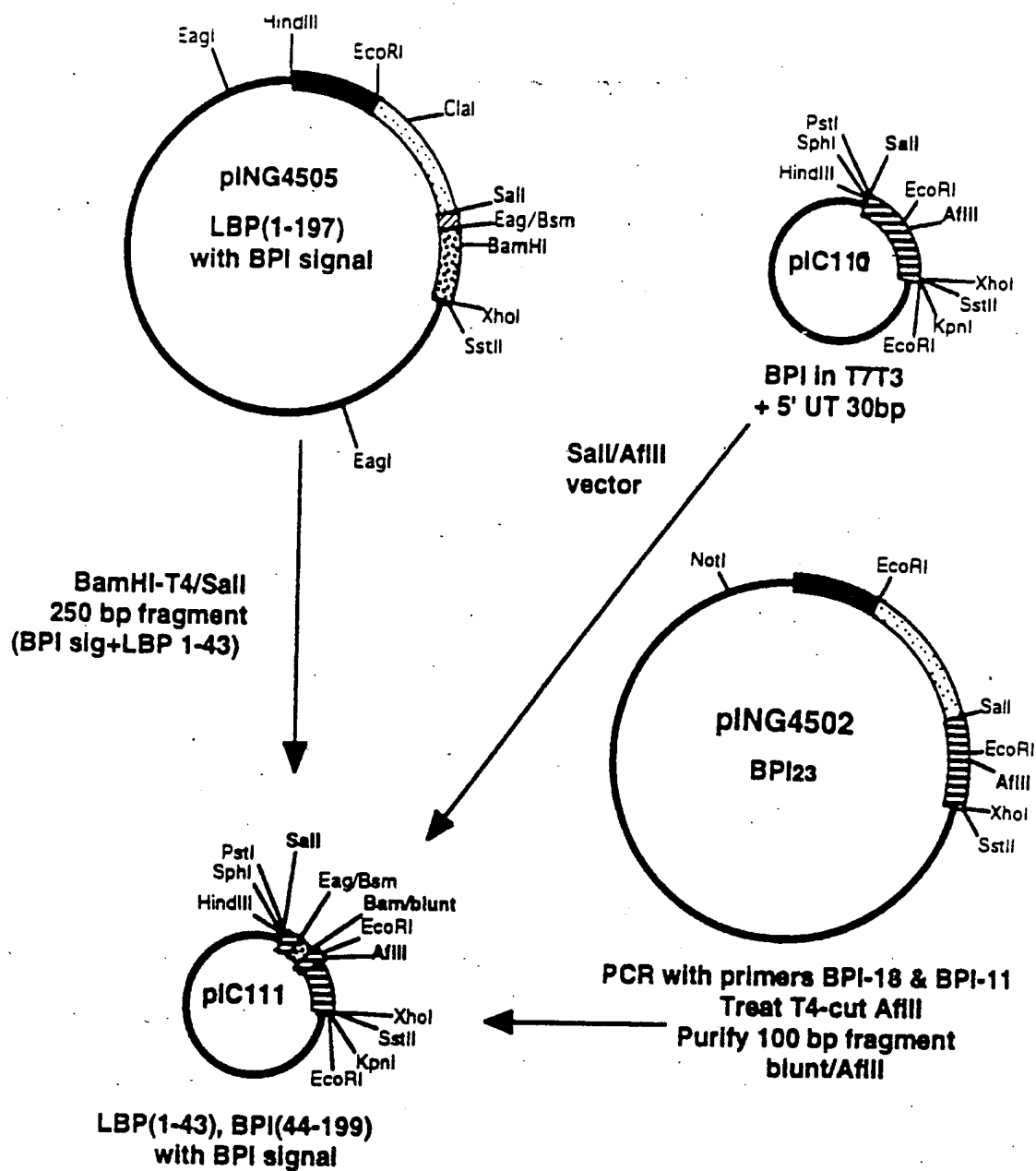
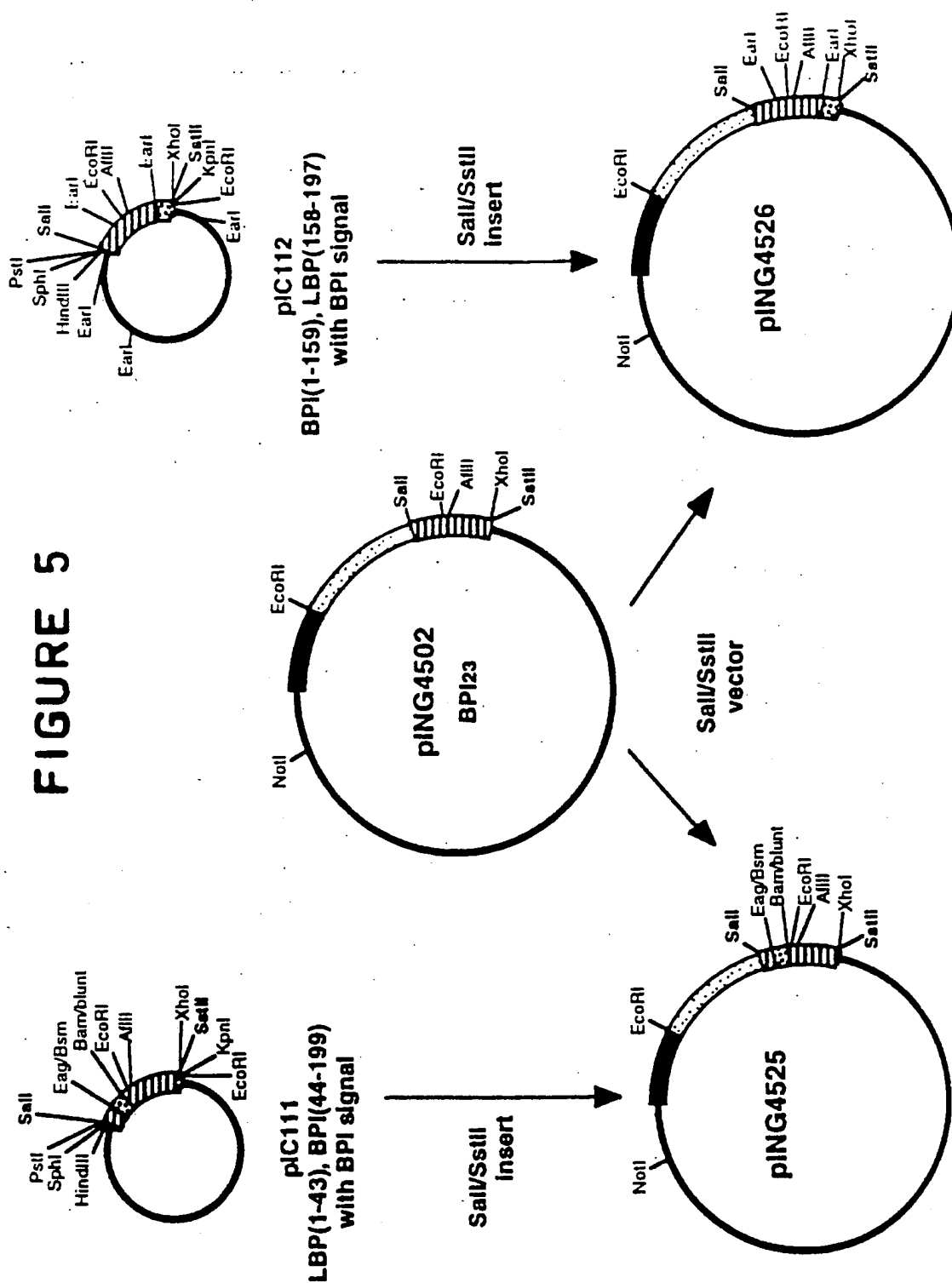


FIGURE 4

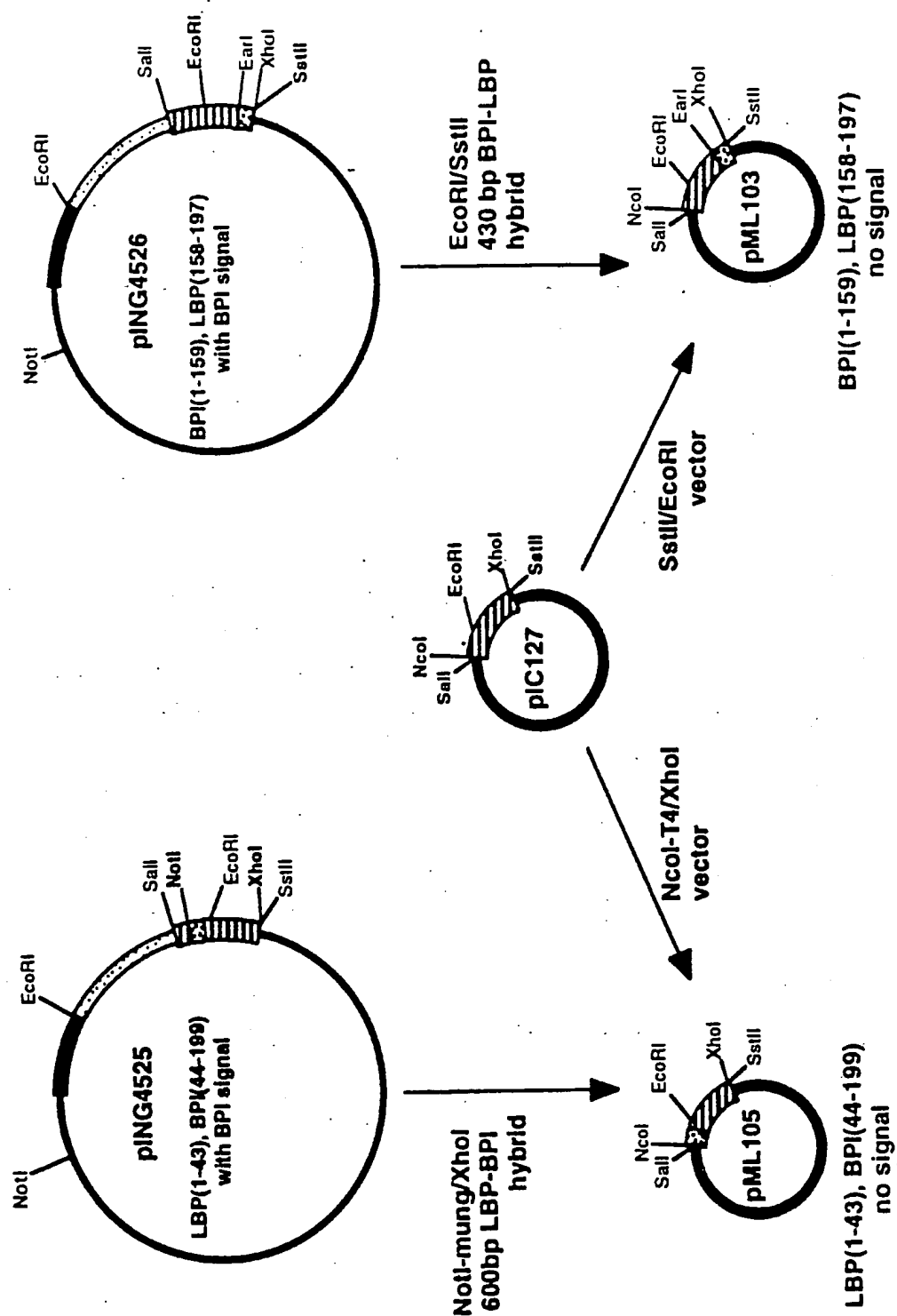
6 / 21



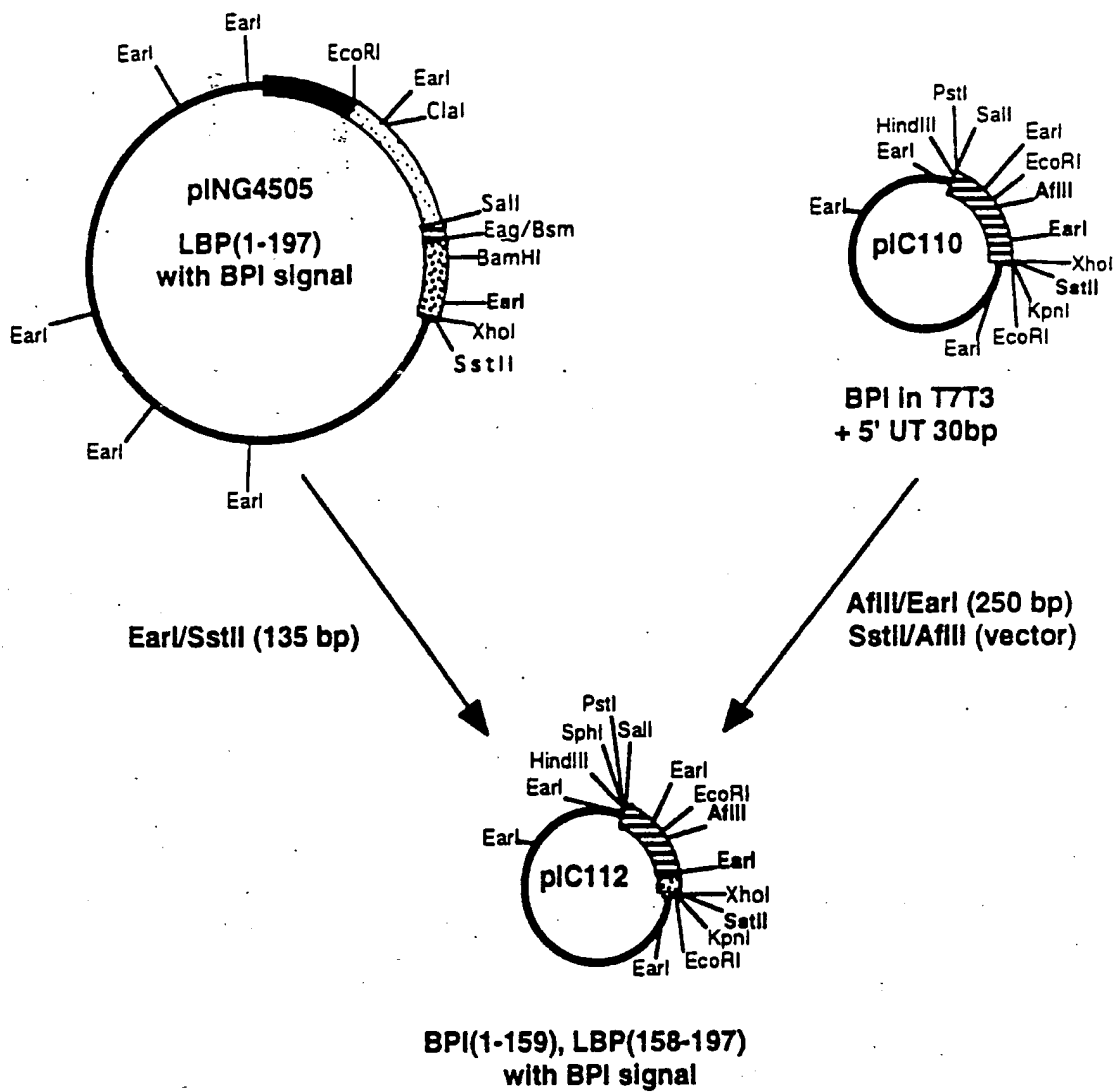
SUBSTITUTE SHEET (RULE 26)

7 / 21

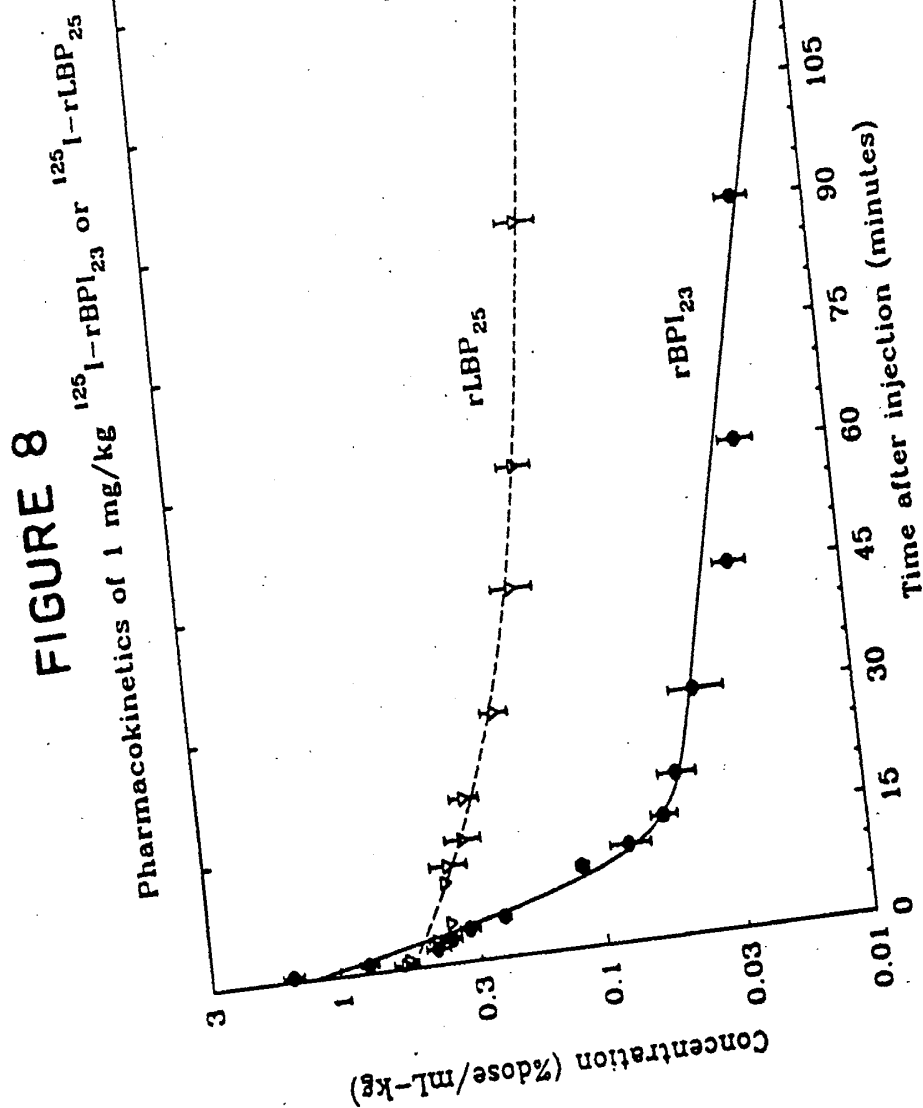
FIGURE 6



8 / 21

**FIGURE 7**

9/21



SUBSTITUTE SHEET (RULE 26)

10 / 21

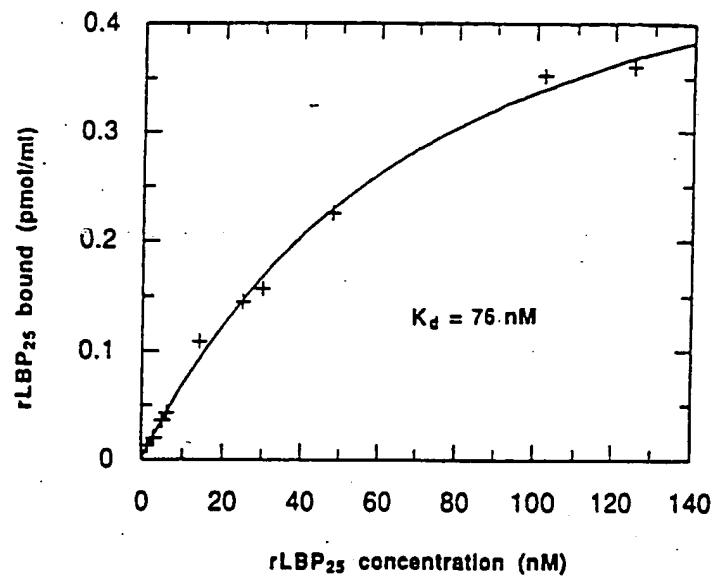


FIGURE 9

11 / 21

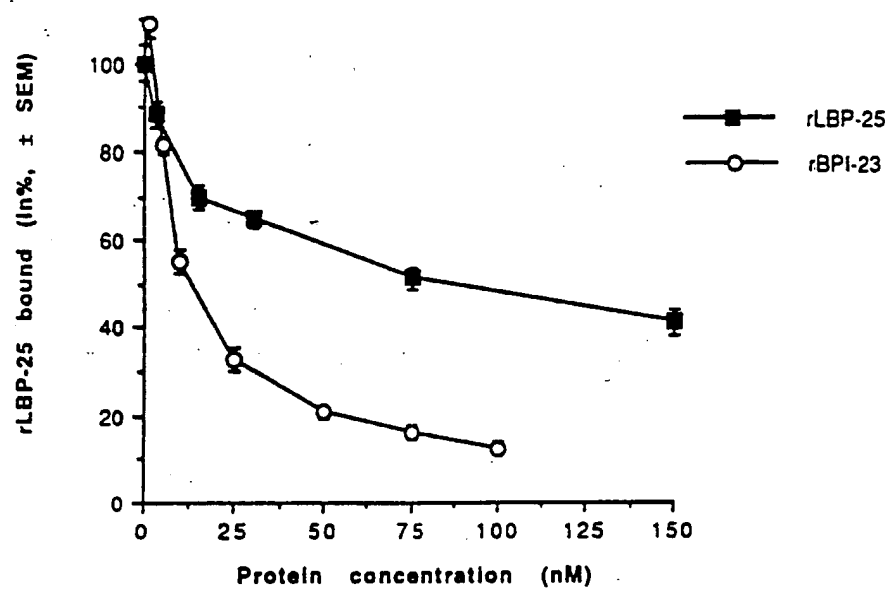


FIGURE 10

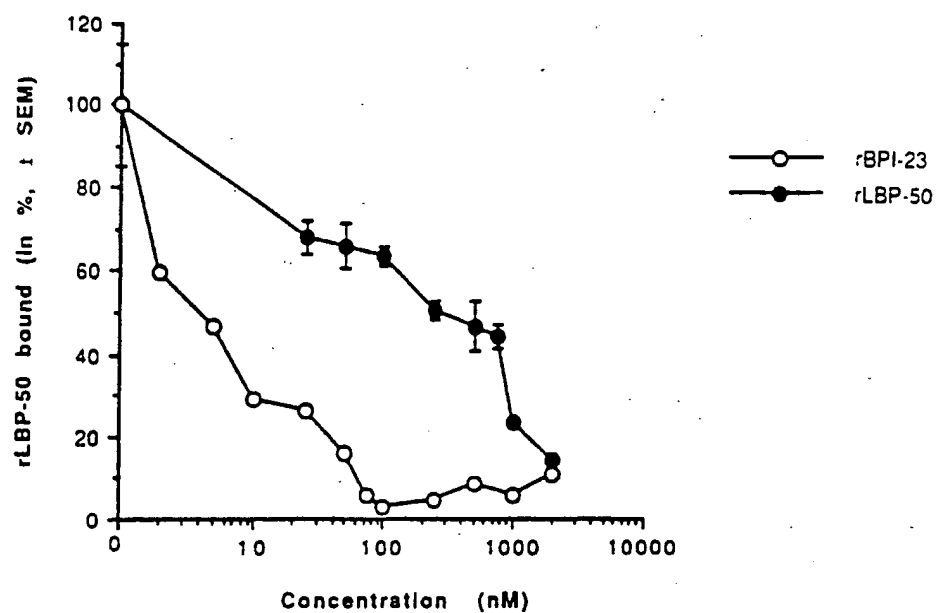


FIGURE 11

12 / 21

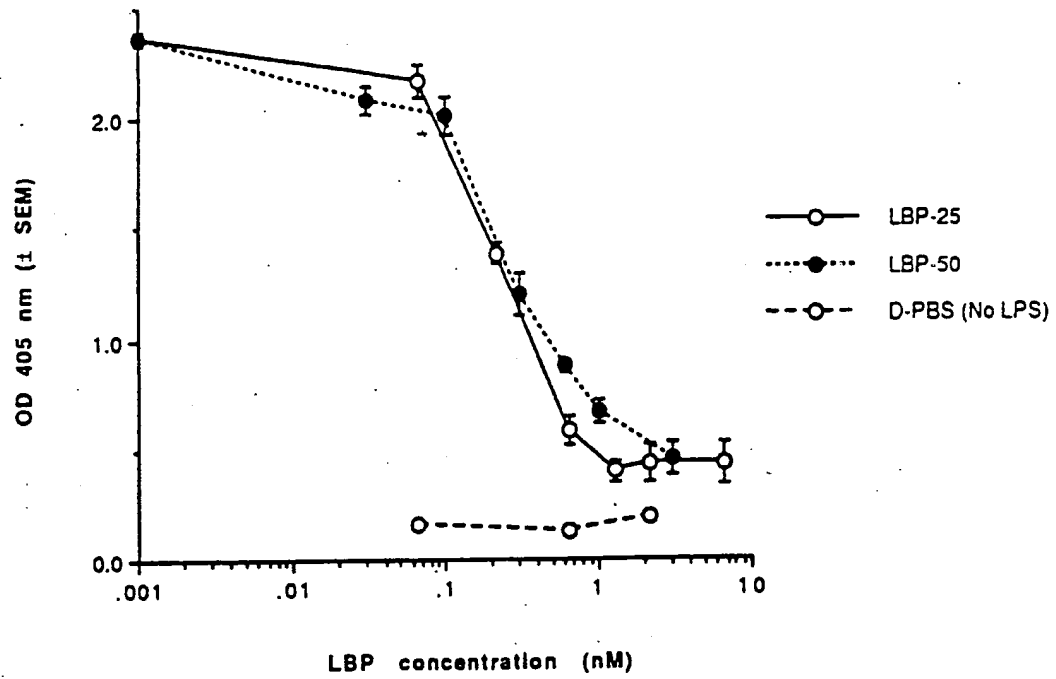


FIGURE 12

13 / 21

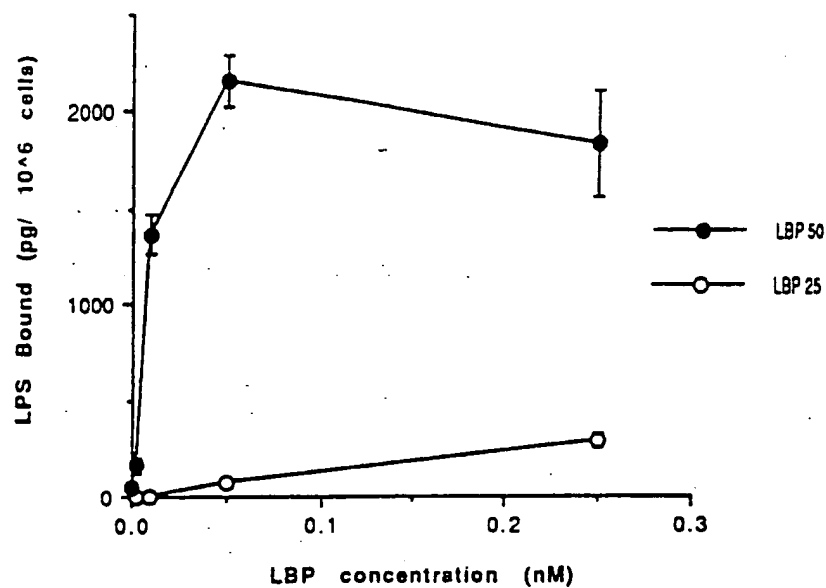


FIGURE 13

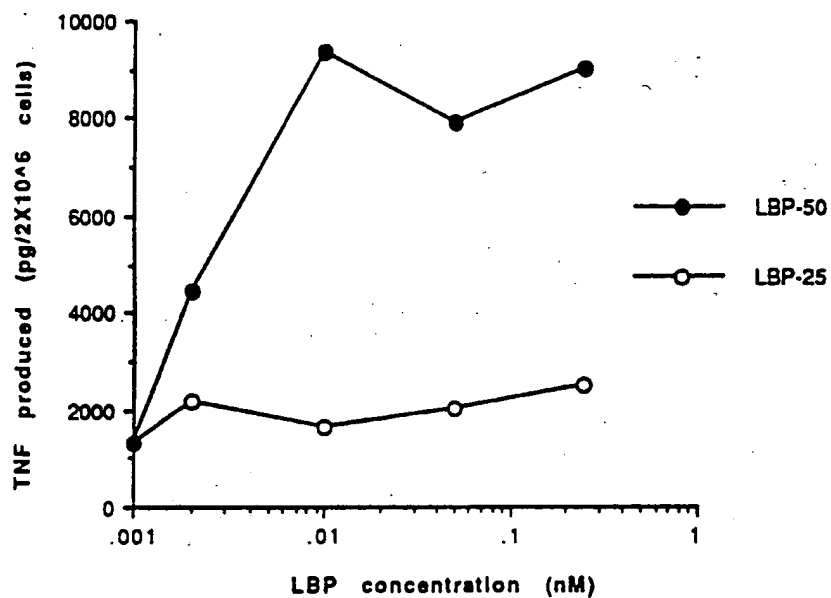
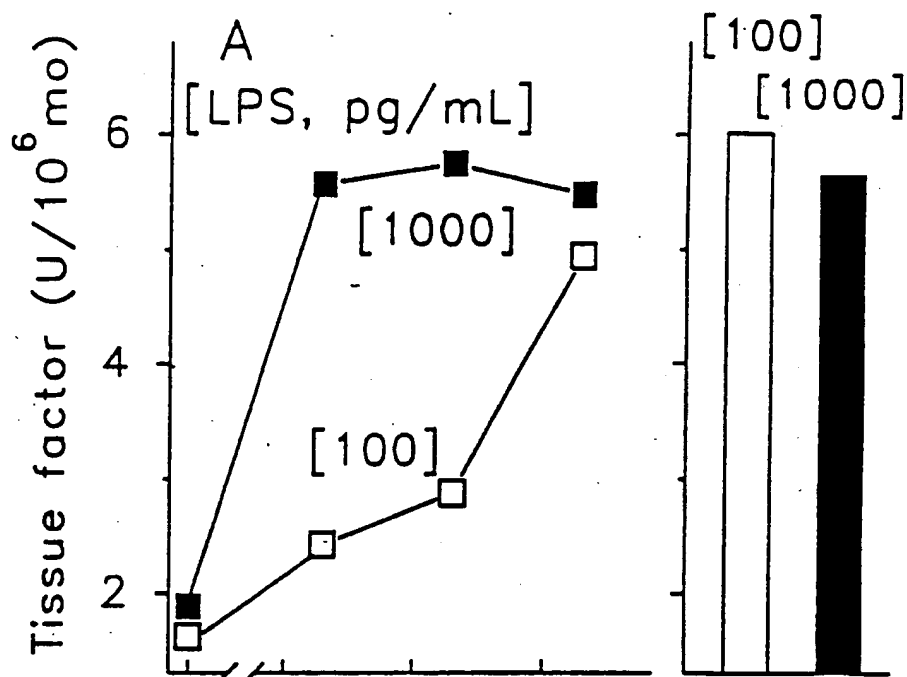
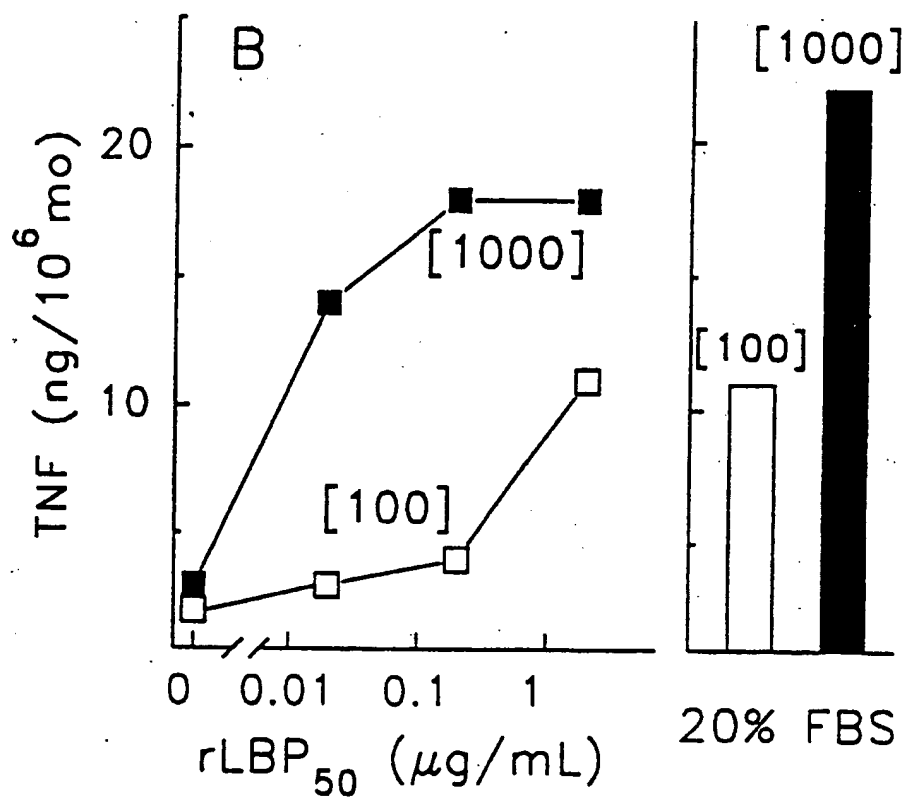


FIGURE 14

14 / 21

**FIGURE 15A****FIGURE 15B**

SUBSTITUTE SHEET (RULE 26)

15 / 21

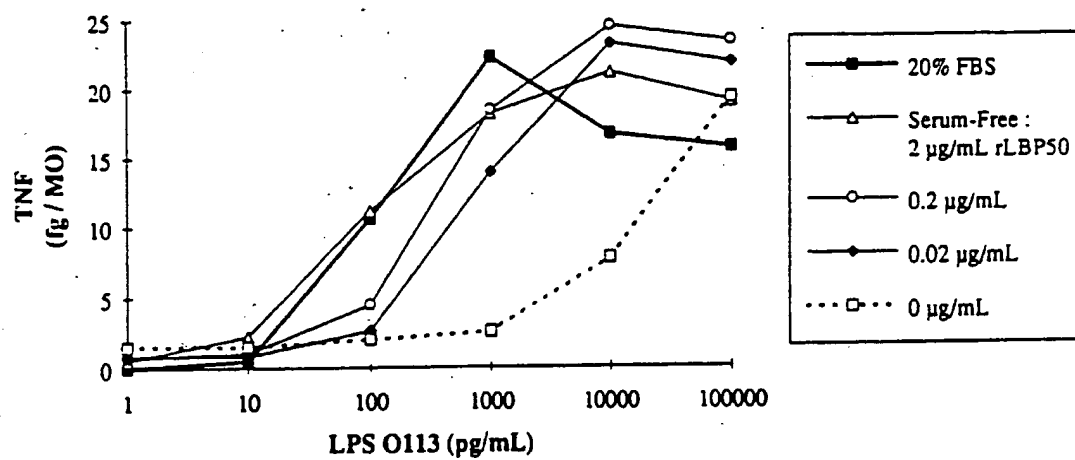


FIGURE 16

16 / 21

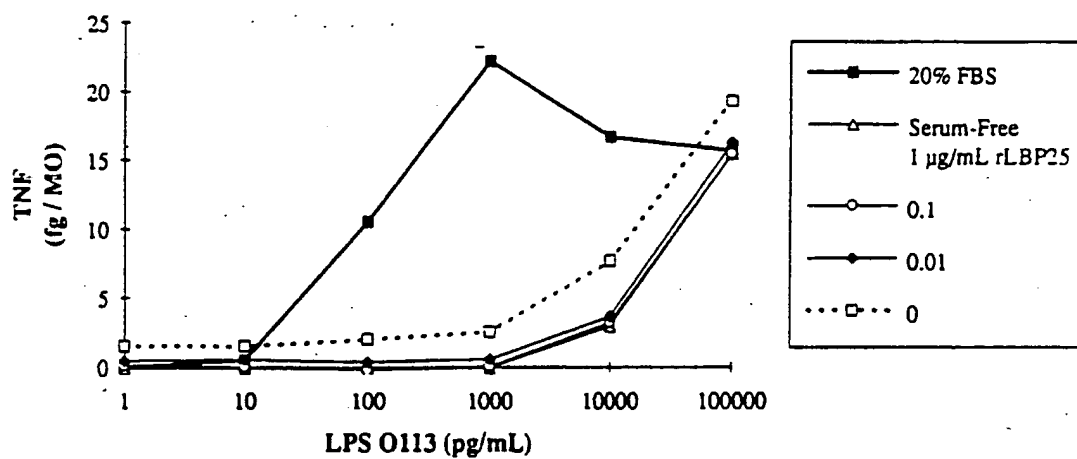


FIGURE 17

17 / 21

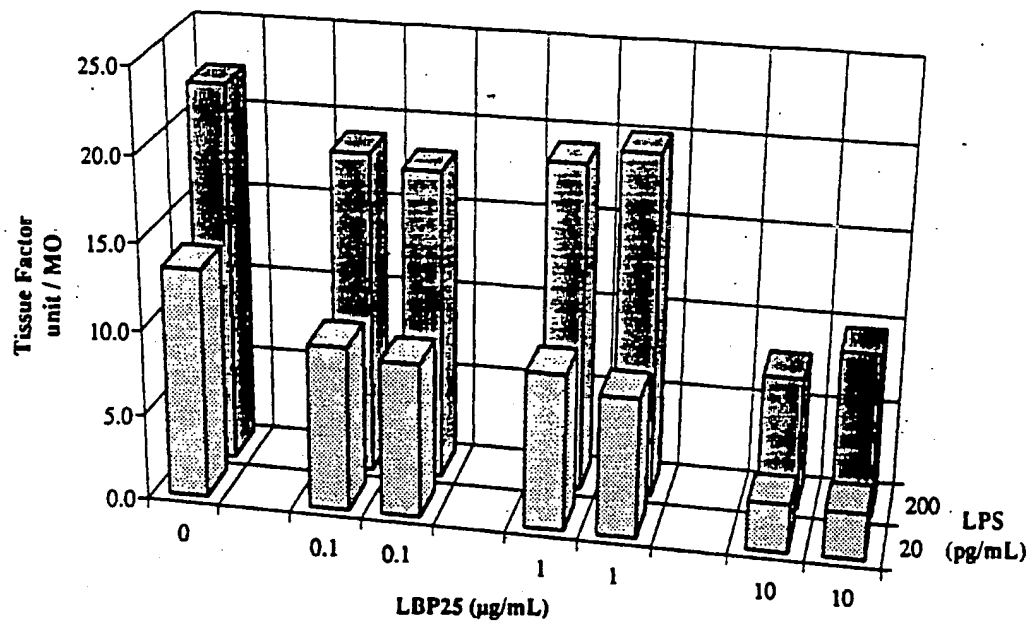


FIGURE 18

18 / 21

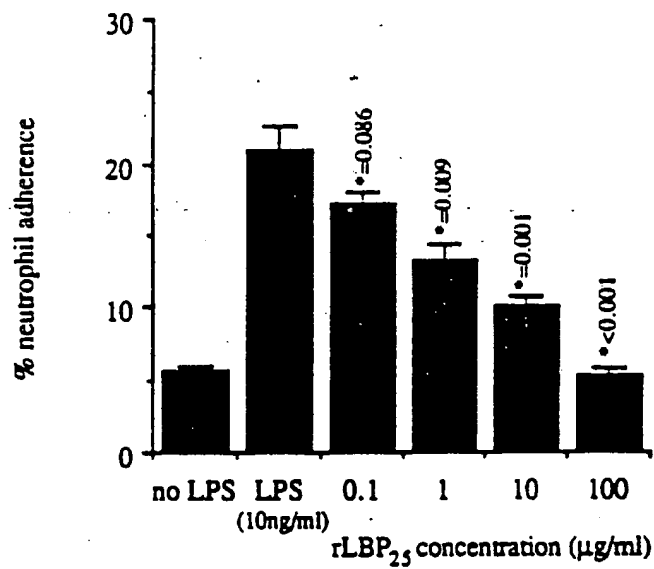


FIGURE 19

19 / 21

FIGURE 20

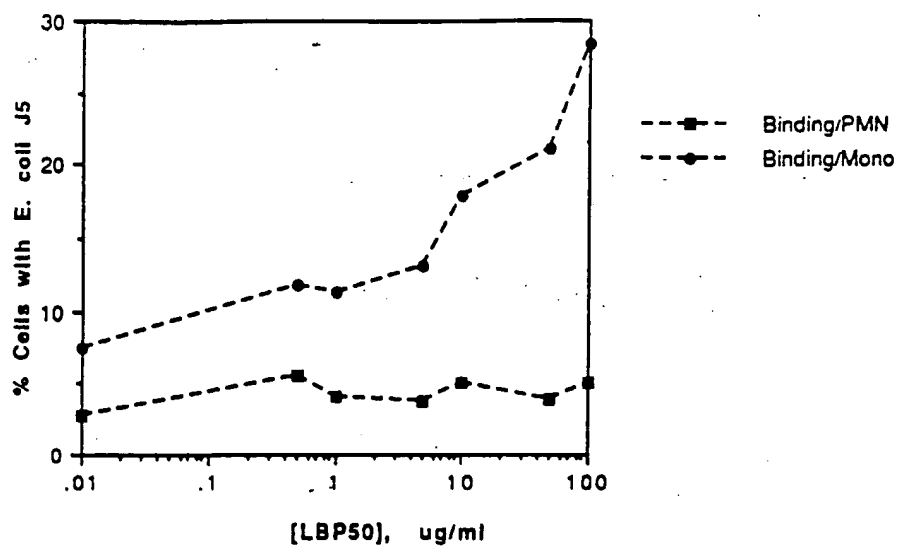
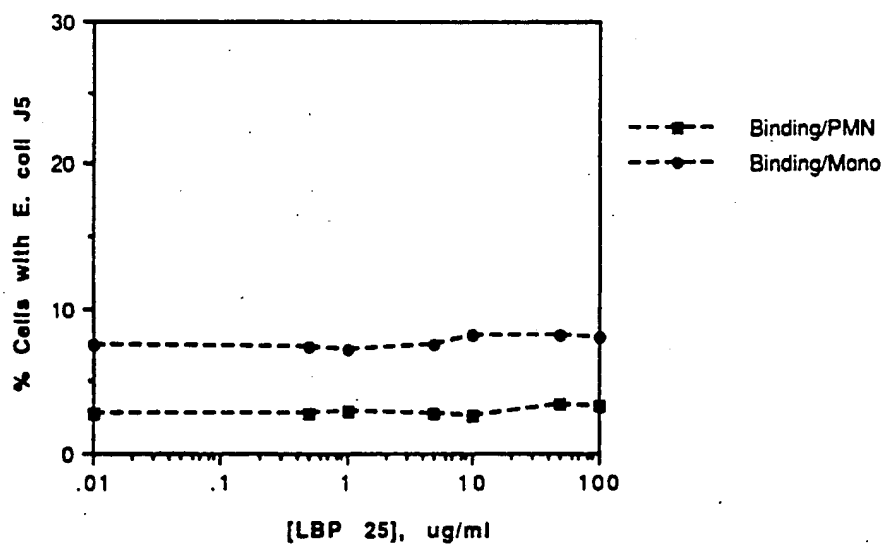
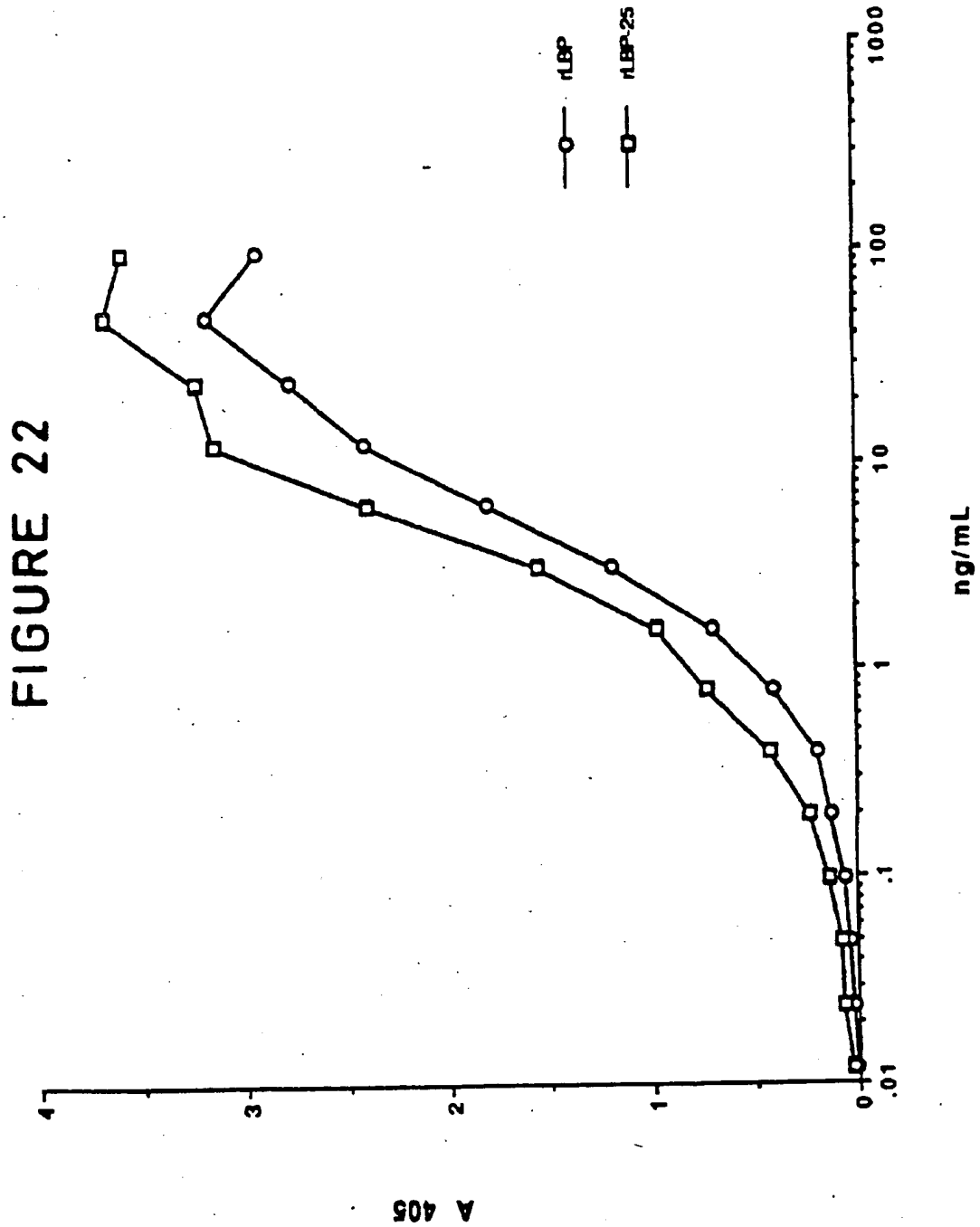


FIGURE 21



20 / 21



21 / 21

BPI23/LBP25 SEQUENCE ALIGNMENT

```

10      20      30      40      50      60
BPI...VNPGVVVRISQKGLDYASQQGTAALQKELKRIPDYSDSFKIKHLGKHVSFYSDIRE
      :: :: :: :: :: :: :: :: :: :: :: :: :: :: :: :: ::
LBP...ANPGLVARITDKGLQYAAQEGLLALQSELLRITLPDFTGDLRIPHVGRGRYEFHSLNIHS
10      20      30      40      50      60

```

```

70      80      90     100     110     120
BPI...FQLPSSQISMVNPVGLKFSISNANIKISGKWKAQKRFKMSGNFDLSIEGMSISADLKLK
      : : : : : : : : : : : : : : : : : : : : : :
LBP...CELLHSALRPVPGQGLSLISIDSSIRVQGRWKVRKSFFKLQGSFDSVKGISISVNLLLG
70      80      90     100     110     120

```

```

130     140     150     160     170     180
BPI...SNPTSGKPTITCSSSHINSVHVHISKSKVGWLIOLFHKKIESALRNKHNVSQVCEKVTN
      : : : : : : : : : : : : : : : : : : : : : :
LBP...SE-SSGRPTVTASSCSSDIADVEVDMSG-DLGWLLNLFHNQIESKFQKVLESRICEMIQK
130     140     150     160     170

```

```

190     199
BPI...SVSSELQPYFQTLPVMTKI
      : : : : : : : : : : : :
LBP...SVSSDLQPYLQTLPVTTIEI
180     190     197

```

FIGURE 23

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/12 C07K13/00 C12N15/62 A61K37/02 C12P21/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07K C12N C12P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 03535 (INCYTE PHARMACEUTICALS, INC.) 5 March 1992 cited in the application see example 5	1-3
A	see figure 23 ----	1-17
A	WO,A,92 09621 (NEW YORK UNIVERSITY) 11 June 1992 see page 8, line 19 - page 10, line 33 see examples 3,4 ----	6-9
P,X	JOURNAL OF IMMUNOLOGY 152 (7). 1994. 3623-3629 THEOFAN, G. ET AL. 'An amino-terminal fragment of human lipopolysaccharide-binding protein retains lipid A binding but not CD14-stimulatory activity.' see the whole document ----	1-4
-/--		



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

5 October 1994

Date of mailing of the international search report

21. 10. 94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Andres, S

INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/US 94/06931

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO/A,93 23540 (XOMA CORPORATION) 25 November 1993 see examples 3,4	1-4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/06931

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 15-16 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 94/06931

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9203535	05-03-92	US-A- 5171739	15-12-92
		AU-A- 8850191	17-03-92
		EP-A- 0544832	09-06-93
		JP-T- 6504267	19-05-94
		US-A- 5234912	10-08-93
		US-A- 5334584	02-08-94
		US-A- 5308834	03-05-94

WO-A-9209621	11-06-92	AU-A- 9127591	25-06-92
		EP-A- 0563222	06-10-93

WO-A-9323540	25-11-93	AU-B- 4382093	13-12-93
